REMARKS

Authorization is provided to charge the fee for filing the RCE, the extension of time and any fees that may be due in connection with the filing of this paper or with this application to Deposit Account No. 02-1818. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

A supplemental Information Disclosure Statement is filed under separate cover on the same day herewith.

An executed Declaration under 37 C.F.R. §1.132 of Dr. Koster is attached. A signed original and a copy are attached. The signed original printed on both sides of the paper by Dr. Koster is provided. To ensure that all pages are scanned, one-sided copy of the signed Declaration also is provided. Attachments reference below and a paper Fischer *et al.* referenced in the Declaration also are provided.

Claims 1, 2, 5, 6, 10, 15, 17, 18, 22, 25, 34, 38, 41, 43, 44, 47, 55, 56, 63, 66-68, 75, 77, 110, 116, 137, 139, 140, 143-147, 151-153, 155-157, 160, 161, 163, 164, 166-169, 171, 172, 174 and are pending. Claims 43, 44, 158 and 159 are cancelled without prejudice or disclaimer.

Claims 1, 2, 6, 10, 46, 163 and 164 are amended, and claim 175 is added to advance prosecution by including claims directed to particular embodiments that are clearly outside the purview of any rejections and amending the claims to address particular rejections. Claim 1 is amended to render it clear that X is a photoactivable group that, upon exposure to light, binds to proteins. Claim 1 also is amended to recite that the drug (fragment, metabolite, intermediate or prodrug thereof) is a small organic molecule drug. Basis can be found, for example, at page 21, which recites:

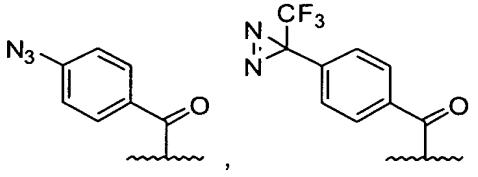
> As used herein, a **drug** refers to any compound that is a candidate for use as a therapeutic or as a lead compound for designing a therapeutic or that is a known pharmaceutical. Such compounds **can be small molecules, including small organic molecules**, peptides, peptide mimetics, antisense molecules, antibodies, fragments of antibodies or recombinant antibodies.

In addition, as discussed below, Applicant respectfully requests examination of all of the claims that read on the elected species (compound A, page 124), in which Q is biotin; Z is an amino acid, lysine; X is photoactivatable, and Y is a user selected pharmaceutical small molecule drug or a fragment, metabolite, intermediate or prodrug of the drug.

The undersigned in drafting this response has endeavored to address the rejections by amendment and/or by the detailed discussion and analysis. The undersigned would be

pleased to discuss this with the Examiner to aid in review of this response. One important distinction to appreciate is that in the instant methods, the functions of capture and interaction are separated (which is why and how targets and non-targets are captured). The method assesses the interactions of drug (or other small molecule) that is presented on a capture compound that includes a non-specifically binding (*i.e.* a group that covalently binds to proteins) activatable group X and a group Q, for sorting or immobilization. In the method, the Y group interacts with all proteins in a sample that can interact with it, and X, when activated, captures all of the proteins that interact with Y. This is contrast to the methods in the cited prior art and other methods, in which the interactions of the X group (the capture group) are employed for specific detection of an analyte. In the instantly claimed methods, the Y group interactions are being assessed, but Y does not capture the interacting proteins, X, which covalently binds to proteins, captures the interacting proteins. This is discussed in more detail below.

Also, while it is believed that all claims are allowable, in an effort to advance at least some claims to allowance, claims, such as claims 2, 6, 46, 163, 164 and 175 and others are amended to recite specific groups for Z, X and/or Q; these claims should be outside the purview of the 35 U.S.C. § 112, first paragraph rejections, since X, Z and Q are recited with particularity. Y is user selected, and, thus, cannot be specified. One would not claim a mass spectrometry method and require a claim to limit the method to only particular proteins (*i.e.*, a mass spectrometric method for identifying cytochrome C).

Specifically, claim 2 is directed to the method of claim 1 where Z comprises an amino acid; and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid (PNA) and an activated dextran (exactly as recited in the specification at pages 122-123 (section 4, which describes Q); see, also page 53, referenced by the Examiner). Claim 44 recites that X is a diazirine, 3-trifluoromethyldiazirine or an azide; Z is an amino acid and Q is biotin. Claim 175 is directed to method of claim 1, where X is  or an arylazide; Z is serine, threonine, lysine, tyrosine or cysteine; and Q is biotin or an

oligonucleotide. Claim 6 recites that X is selected from among an azide or a diazarine; Z is an amino acid; and Q is biotin or an oligonucleotide.

Claim 43, recites the method of claim 34, which defines Z, and states that M is an amino acid; S^1 and S^2 each is independently $(CH_2)_r$, where r is 1-10; and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid and an activated dextran. Claim 139 recites the method of claim 137, where M is an amino acid; and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid and an activated dextran; and claim 140 recites that X is an azide, S^1 and S^2 each is independently $(CH_2)_r$, where r is 1-10, and Q is biotin or an oligonucleotide.

Thus, these dependent claims recite specific groups for each of X, Z and Q. Y is as user selected drug whose interactions are of interest. It makes no sense to limit Y to the drugs discussed in the specification to exemplify practice of the method. It makes no sense to limit the claims to particular drugs, since the method is for assessing interactions of drugs per se, and once the method is practice with one drug, one is done. It makes no more sense to limit the claims to particular drugs, than it does to limit methods of drug discovery to specific drugs, or methods of screening to screening particular compounds. As pending, the claims recite that the drug is a small organic molecule drug. One of skill in the art can readily select small molecule organic drug of interest, just as Dr. Koster selected the Parkinson's drugs described in the Declaration for investigation. The instantly claimed methods are for assessing the interactions of small molecule drugs with non-targets.

OBJECTION TO THE CLAIMS

Claim 2 is objected to as being an improperly dependent on claim 1. Without addressing the propriety of this objection, it respectfully is submitted that amendment of claim 2 herein renders this object moot.

PRIORITY

The Examiner's comments with respect to according priority to the claims are noted.

INFORMATION DISCLOSURE STATEMENTS

A supplemental Information Disclosure Statement updating prior Information Disclosure Statements and addressing the issues raised in the Office Action is filed under separate cover on the same day herewith.

ELECTED SPECIES AND CLAIMS THAT READ ON THE ELECTED SPECIES

Applicant elected methods in which the capture compound used in the methods is compound A, which is used in the Examples to demonstrate that the capture compounds interact with targets and non-targets as predicted for a known drug, a sulfonamide, whose targets and non-targets are known. In compound A, Q is biotin, X is a photoactivatable group, aryl azide, Y is a user selected drug, which in the Example is a sulfonamide, and Z is an amino acid, lysine. An amino acid is **not cleavable** by mass spectrometry. For example, at page 70, lines 2-5, the specification describes exemplary non-cleavable moieties Z with the formula (S1)tM(R15)a(S2)b, where t and b are each independently 0 or 1 and a is 0 to 4 (see, e.g. page 60, lines 18-23). If t, b and a are all 0, then Z is a non-cleavable moiety with formula M. The specification describes that exemplary structures of formula M for non-cleavable Z moieties, include amino acids (see, e.g. page 60, line 31 through page 61, line 1; and page 63, line 2, which recites that exemplary M structures include (NHCH(R52)C(=O), where R52 is the side chain of a natural or unnatural amino acid). Therefore, the specification describes that amino acids such as lysine are exemplary noncleavable moieties Z. Thus, amino acids such as lysine are not cleavable moieties Z, because conditions required for cleavage of an amino acid would result in cleavage of proteins, since proteins are composed of amino acids.

Therefore claims that read on the elected species are mis-identified in the Office Action. Claims, such as claim 17, which recites that which Z is not cleavable by mass spectrometry, read on embodiments in which Z is an amino acid do read on the elected species.

For clarity, the currently pending claims included among those that read on the elected species are claims: 1, 2, 5 (see below), 6, 10, 17, 18, 25, 34, 43, 46, 47, 63, 66, 67, 68, 75, 110, 116, 137, 139, 140, 151-153, 160, 161, 163, 164, 166, 169 and 175. Accordingly, these claims should be examined.

Regarding claim 5, which recites that Y is presented on the compound in different orientations, the Examiner urges that the elected species does not read on it, since the elected species is not presented in different orientations. If the Examiner's approach is adopted, as set up by the Office, there is no species that could have been elected on which this claim

reads, since it reads on methods in which the drug, Y, is presented in different orientations on the capture compound. As set forth, the election of species requirement, required election of a method in which the capture compound used in the method is a single compound. A method in which the capture compounds present Y in different orientations requires a plurality of compounds. Applicant respectfully submits that a method in which a plurality of capture compounds are used in the method encompasses the elected species in which the orientation of Y is not specified. It also encompasses the elected species if one of the capture compounds reads on the elected species. Thus, claim 5 should not be withdrawn.

DESCRIPTION OF THE CLAIMED SUBJECT MATTER AND DECLARATION OF DR. KOSTER

A. The claimed subject matter

In an effort to advance prosecution, claims, such as claims 2, 6, 43, 44, 137, 139,140 and 175 are amended so that X, Z, and Q are limited to a few groups (those used in commercial embodiments). Y is a user selected drug (or fragment, prodrug or other form thereof), whose interactions are of interest.

The methods of these claims should be outside the purview of the rejections under 35 U.S.C. §112, first paragraph, because the structure of the capture compound is provided and the identify of all groups is specified. Also, all pending claims are clearly different from the cited art; not only do the methods differ from the cited art in that non-targets (molecules with which the drug of interest interacts and can be responsible for undesirable side effects), the capture compounds do not resemble any compounds in the cited art, which provides a polypeptide linked to biotin and containing an amino acid ligand in the middle of the polypeptide that specifically binds to guanyl cyclase. Thus, Applicant respectfully requests consideration to advance at least these claims to allowance.

In addition, to aid in the following is a discussion of the claimed method as described in the application (which describes other methods as well). The claimed method is unlike any method in the prior art. The method is depicted, for example, in Figure 30. The method, as claimed, is for assessing interactions of a small molecule (Y), such as a drug to indentify what targets and, particularly, non-targets of Y in a sample with which Y interacts. Targets are those biomolecules with which, for example, a drug is intended to interact, and non-targets are other biomolecules with which it also interacts. As amended, the claimed embodiments recite that the biomolecules are proteins. The methods provide a way to present the drug to identify all biomolecules (proteins as presently pending) in a sample with

which the drug interacts. A drug will interact with its intended target, but also, interacts with other molecules, which interactions can be responsible for undesirable side-effects. The Declaration of Koster and attached reference are provided to illustrate the power and elegance of the method when applied to a drug that was known to have toxic side-effects on the liver. The Declaration describes experiments in which the non-targets are identified. From the identity of the non-targets the causes of the side-effects were deduced. The interactions of the capture compound with an hepatocyte lysate with the toxic drug is compared to capture compound that presents an improved version of the drug. It is shown that the improved drug interacts with fewer non-targets and does not interact with those responsible for the toxicity.

To practice the method, the Y molecule, which is a small molecule of interest (in the claimed embodiments, Y is a drug or a fragment, prodrug, intermediate or metabolite of the drug), is presented on a capture compound, which includes an activatable moiety X for capturing biomolecules that are interacting with Y, and a sorting function, Q, for immobilization or detection. X, Y and Q are presented on a trifunctional core Z, which is selected to present X, Y and Q and to present X and Y such that, when activated, X captures the molecules that are interacting with Y. Z moieties, with amino acid side chains, such as amino acids, are exemplary of suitable Z groups. In the elected species Z is lysine. In practicing the claimed method, the capture compound that presents the Y molecule is contacted with a sample, such as a cell lysate, and Y, the presented drug, is allowed to interact and come to equilibrium with proteins in the sample. X is then activated and it captures proteins that are in the vicinity of Y. These molecules include any with which Y interacts, including the drug targets, but also non-targets.

The pending claims are directed to the **methods**, not to capture compounds. While compounds described in the application are novel, capture compounds with X, Z and Q groups are known in the art and also are described in detail in the application so that one of skill in the art could readily select appropriate X, Z and Q moieties. Y is user selected; the application teaches a variety of appropriate Z groups (*i.e.* trifunctional groups) for presenting Y along with X and Q. Q, which is group for immobilizing the compounds are well-known, and also described in detail in the application. X is a photoactivatable group that binds to proteins. Such groups are well known in the art. Thus, the instant claims, however, do not rely on the novelty of the compounds in the methods for patentability. Rather the steps of the method, and hence, the method, which identifies non-targets for a drug is novel.

The methods provide a new way of using capture compounds as vehicles to present a drug or other small molecule of interest, and a new method to determine with what a small molecule, Y, which is presented on the capture compound interacts. Furthermore, the outcome of the method, identification of any non-targets of Y in sample with which Y interacts, is not taught or suggested by any art of which Applicant is aware.

Generally in prior art methods that use capture compounds, the capture compounds are reagents used to detect a particular analyte or to capture a targeted compound; the capture compounds are **not** the analyte of interest. The interactions of the capture compound are not assessed. For example, in the cited reference, Hasegawa *et al.* a photoaffinity labeled ligand, that also includes biotin, is employed as a reagent to specifically bind to the target site for the ligand. The compound of interest is not enterotoxin, but the guanyl cyclase.

In the instant method, the capture compounds **are the analyte of interest,** the interactions of the capture compound are assessed (since the compound presents the small molecule whose interactions are to be assessed). This is an important distinction and the pending claims capture this distinction, such as by reciting that a capture compound that presents the drug (or a fragment, metabolite, prodrug thereof), is provided and contacted with a sample sufficiently long for the drug portion of the compound to interact with all molecules in the sample with which it can interact, then the X group is activated molecules that interact with Y are captured, and non-targets are identified or isolated.

In the claimed method, the use of capture compounds, simplistically, can be compared to a Venus fly trap, where the drug or other small molecule, whose interactions are to be assessed (the "Y" group) is sweet smelling nectar that attracts bugs to the Venus fly trap. The bugs include good bugs (the drug targets), but also bad toxic bugs (the non-targets). The Venus fly opens and bugs (good, toxic, and others are flying around). The good and toxic bugs are attracted to the nectar ("Y") and then after enough time for good and toxic bugs to get near, the trap slams shut (X is activated) and captures the good and toxic bugs. Both good and toxic bugs are then identified.

As discussed in previous responses and above and below, prior art methods, do not present a compound of interest on a capture compound, and prior art methods do not assess what non-targets (toxic bugs) interact with a drug or other user selected small molecule of interest. Also, prior art methods target particular analytes and are not designed to capture analytes and non-targets. Furthermore, as discussed below, the instant claims are directed to methods that employ a capture compound that presents the user selected Y group; capture

compounds with activatable capture groups and sorting functions to permit separation of the capture compounds are well known. In addition, as discussed in previous responses, the application describes X, Z and Q groups for use in the capture compounds in described in great detail in the application.

Thus, the claims are directed to methods for assessing the interactions of small organic molecule drugs or fragments, metabolites, intermediates or prodrugs thereof that can interact with non-target with which the drug interacts in order to identify the molecules in a sample that interact with the small organic molecule drugs or fragments, metabolites, intermediates or prodrugs thereof. Among the molecules in a sample that interact with small organic molecule drugs or fragments, metabolites, intermediates or prodrugs thereof are those that are the targets for the drug and also those the are not targets (non-targets). Targets are the molecules whose actions are intended to be altered by the drug. The non-targets, whose definition the Examiner discussed in the Office Action, include molecules that contribute to or cause undesirable side effects by virtue of their interaction with the drug.

The method is practiced by contacting the capture compound. which presents the small organic molecule drugs or fragments, metabolites, intermediates or prodrugs thereof as "Y" and that includes the X group, with a sample for a sufficient time for interactions with Y to reach equilibrium under conditions in which X is not activated, and then activating X (venus fly trap shutting) to capture anything (the good bugs (targets) and toxic bugs (non-targets)) that is interacting with Y, thereby identifying any molecules in the sample with which Y interacts. One then can identify these molecules, and, from among them identify the molecules that are non-targets and that can be responsible for drug side-effects. By including Y on the capture compound and allowing the reaction to proceed to equilibrium, any biomolecule, such as a protein, in a sample that can interact with Y will do so and will be captured upon activation of X.

The methods in this application permit identification of any biomolecules in the sample that interact with the drug. The molecules that interact with Y include any targets for such molecules, and also any non-targets that are present in a sample. In general, the target of a drug is known, but it is not necessary to know it to practice the method, as targets and non-targets are captured. *In vivo*, undesirable side-effects, often are caused by or related to interactions with non-targets. Hence, identification of such non-targets can provide insight into side-effects and also, for example, permit redesign of drugs to be more target-specific. The methods in this application permit screening for binding to non-targets and then selection

of drugs or other molecules that bind to fewer non-targets or redesign of the drugs to bind to fewer non-targets.

Description of the claimed method in the application

The application provides a detailed description of the X, Z and Q moieties and provides details regarding practice of the method at, for example, Section 6, at pages 151-154, page 92- page 93, page 94, line 29, - page 102, in Example 16, in Figures 30-36, and elsewhere in throughout the specification. The discussion in the sections of the response describing the teachings of the specification in connection with the rejections under 35 U.S.C. §112, first paragraph, provide a summary of some of this disclosure. Since the application describes other methods in addition to those presently claimed, this discussion is repeated here to describe where in the application the claimed subject matter is described.

The description in the specification and figures provides details and working examples of the claimed methods. The method includes the steps of: (1) contacting a capture compound that presents a user-selected Y group (small molecule, such as a drug) with a sample for a sufficient time for the interaction between the Y group and the biomolecules to reach equilibrium; (2) activating X to form a covalent linkage with the biomolecules with which Y interacts; and (3) identifying captured molecules to identify molecules that interact with Y. The purpose of the method is to identify any molecules, particularly those that are not the targets for Y, in a sample that interacts with Y. Y is chosen by the user and is a compound, such as a drug, or portion thereof, whose interactions are to be assessed. It is not necessarily known whether any molecules in a particular sample will interact with a particular Y.

Y is presented on capture compound, which includes a moiety X that, upon activation, covalently binds to biomolecules, contacted with a sample sufficient for any interaction with Y reach equilibrium. X forms covalent linkages with biomolecules interacting with Y. Thus, one can probe a sample to see what molecules interact with Y. If Y is a drug, with a known target, it should interact with its known target. Also, drugs and other small molecules interact with other biomolecules in addition to their intended target. In this method, any biomolecules with which Y interacts are captured and can be identified. Such molecules could be responsible for side-effects of the drug. As described in the application, the drug can be redesigned so that it does not interact with these non-drug target molecules.

Hence, Y is selected by the user of the method. X is described in detail in the application and is any of the many well-known activatable moieties that interact with

biomolecules, particularly amino acid side chains that might be in the sample whose interactions with Y are to be assessed. X moieties are described in detail starting on page 79 of the application. The present claims are limited to photoactivatable X groups that upon activation form covalent bonds with biomolecules, particularly proteins. Activatable X groups that react with side chains of proteins and other biomolecules are well-known components of capture compounds known in the art. It should be appreciated that the instant claims are to methods, not to capture compounds, and employ capture compounds that are modified to present Y, a drug or other moiety, whose interactions are to be assessed.

The method of claim 1 does not require that the property and/or structure of biomolecules is known before they are captured. In fact, the purpose of the method is to assess and discover with what "Y" interacts. The method is for assessing with what a particular drug (or fragment, prodrug, metabolite or intermediate thereof), interacts in a particular sample. If Y is a drug, it typically has a target. If Y interacts with molecules in addition to the target, identification of non-targets can aid in identifying sources of side-effects. The method also can help identify targets of drugs, where the target is not known. A purpose of the method is that is that it identifies biomolecules (proteins as presently claimed) in a sample that interact with Y; these will include the targets of Y and non-targets. Sections 6-7, at page 151 through page 155, line 21, the application describes the claimed method:

> **6. Identification of non-target biomolecules**
>
> Many pharmaceutical drugs have side effects that may arise from the interaction of the drugs, drug fragments, drug metabolites or prodrugs with drug non-target biomolecules under physiological conditions.
>
> For example, aspirin reacts with the non-target Cox-1 receptor resulting in side effects such as gastrointestinal toxicity, ulceration, bleeding, perforation of the stomach, liver necrosis, hepatic failure, renal necrosis and possibly stroke and heart attack. Selective Cox-2 inhibitors such as Cox-2 inhibitors such as 4-[5-(4-methylphenyl)-3- (trifluoromethyl)-1H-pyrazol-1-yl] benzenesulfonamide, (Celebrex®) or 4-(4-(methylsulfonyl)phenyl)-3-phenyl-2(5H)-furanone (VIOXX®) have side effects that may be the result of interaction of the drug with non-target biomolecules. As another example, the thaizolidinedione (TDZ) class of antidiabetic drugs are PPAR-g activators. The PPAR-g protein is a receptor important in the regulation of genes involved in the metabolism of glucose and lipids. TDZs are prescribed to diabetic patients in whom blood sugar (glucose) is not properly metabolized. However, TDZ's are known to also interact with PPAR-a, a protein with a similar structure involved in the synthetic pathway of triglycerides, known to be associated with cardiovascular disease. The TDZ Rezulin was withdrawn from the market due to liver toxicity, and Actos and Avandia were recently reported in a Mayo Clinic study to have cardiovascular side effects.
>
> Drug metabolites can also cause toxicity. There are several enzymatic systems responsible for drug metabolism. One such important system is the Cytochrome P450 family, primarily located in the liver. These proteins work by attaching functional

groups to the (usually lipophilic) drug molecules. These functional groups subsequently allow other enzymes to conjugate moieties (glucuronidation, sulfation, etc.) to the metabolites rendering them water-soluble and thus facilitating excretion. Toxicity can occur if a polymorphic form of an enzyme involved in the metabolism malfunction, or a metabolite irreversibly inactivates a cytochrome p450 (suicide inhibition), compromising its excretion potentially leading to a toxic accumulation in the liver. Depending on the presence of these metabolizing enzyme systems in e.g. kidneys, lung, or heart, similar drug toxicities can be observed in those organs.

The capture compounds/collections thereof provided herein, can be used to identify the drug non-target biomolecules that interact with the pharmaceutical drugs/drug fragments, drug metabolites or prodrugs including but not limited to, receptors and enzymes. The identification and characterization of the drug interacting proteins can also lead to unexpected alternative pharmacological benefits. It is not unlikely that drug targets in other unexpected biological pathways would be found, which allow the application of the drug to treat other diseases. A failed drug that might not be efficacious (or too toxic) for one disease could be turned into a blockbuster for another disease.

In one embodiment, the capture compounds/collections thereof are designed to contain pharmaceutical drugs/drug fragments, drug metabolites or prodrugs as the selectivity function and suitable reactivity and sorting functionality. In the methods provided herein, the capture compound/collections thereof are allowed to interact with a mixture of drug target and non-target biomolecules, including but not limited to, receptor proteins. The captured biomolecules are then analyzed to identify drug target and non-target biomolecules. Screening and identification of drug non-target biomolecules can help in understanding side effects of the pharmaceutical drugs and permit modification of the drug structure to eliminate or minimize the side effects while maintaining the efficacy. Exemplary drug molecules that can be used in the methods and collections provided herein are set forth elsewhere herein, and include, but are not limited to,.LIPITOR® (atorvastatin calcium), CELEBREX® (celecoxib), VIOXX® (refecoxib) and BAYCOL® (cerivastatin sodium).

Once a protein is identified to interact with the drug, public databases annotating the function of many proteins are queried to determine if that structure is likely related to the observed side effect or therapeutic response. For cases where the function of a protein is unknown, bioinformatics and functional genomic tools are available. These include *in silico* approaches (bioinformatics) including sequence alignment, pharmacophores, homology models and protein motif correlation; *in vitro* approaches including liver midrosomes metabolic pathways (e.g. P450), cDNA-expressed enzymes, signal pathways and back-mapping to yeast pathways, simulations and protein/protein interaction of pull-out proteins; *in vivo* approaches including native polymorphisms, knock-out/knock-in, flow cytometry, therapeutic activity of the drug (i.e. therapeutic profile and experimental toxicity, and prospective genotyping and prospective phenotyping. Using these in conjunction with cell-based assays and ribozyme-based knock-in / knock-out technology, which of the proteins identified above are associated with the therapeutic or toxic effect can be determined.

7. Drug Re-engineering

An important goal of most drug development projects is to maximize the interaction between a drug and its target leading to positive therapeutic results, while minimizing interactions with other proteins. Interactions with proteins other than the intended target can trigger a cascade of cellular events leading to side effects. Provided herein are methods that enable design of drugs which interact with their intended target while minimizing other interactions. Here, the selectivity function of the capture compound is a drug molecule or one of its metabolites, attached in different chemically relevant orientations. Following the procedures described above, the proteins (target and non-targets) that interact with the drug and their respective putative function are identified, screening against all cell types potentially involved in the therapeutic or side-effect-related pathways. Knowledge of the therapeutic effect of the drug, as well as its side effects as previously observed in patients, facilitate the formation of a

hypothesis as to which of the captured proteins lead to the desired therapeutic effect, and which are involved in its side effects.

Using these methods, one can iteratively optimize, or re-engineer, the chemical structure of the drug, maintaining or enhancing the desired target protein interactions and eliminating structural features leading to the non-target interactions. Since this process can take place even before preclinical trials, significant cost and time savings can be achieved. The result is a different and patentable new chemical entity (NCE), which can be re-introduced into clinical trials. A reduction of clinical trial time can be envisaged since efficacy data from the related parent drug molecule is already available, and the NCE has been structurally optimized for reduced side effects prior to entering the clinical trail process. An increased success rate of clinical trials would have a tremendous effect on reducing the time and especially the cost of drug development.

Using these methods, analysis is performed to identify the sets of all proteins interacting with the drug, and downstream cellular (functional) assays are used to validate which protein interactions are most likely responsible for the side effects. The drug compounds are redesigned considering data from all the drugs tested in the disease area to maintain the interaction with the protein leading to the positive therapeutic effect while minimizing other protein interactions.

Exemplary diseases that may be studies using these methods include:

(1) Diabetes. Diabetes and its major risk factor obesity will be a growing health crisis facing the western population in the coming decade. Rezulin (Troglitazone) has been withdrawn from the market, MK-767 was recently withdrawn from Phase III trials, and sales of other drugs (e.g. Actos, Avandia) have been hampered, all due to side effects.

(2) Cardiovascular. Nearly one million Americans die each year from cardiovascular diseases, many from heart attacks and strokes due to blocked arteries caused by elevated levels of cholesterol in the bloodstream. However the prescription rate of the statins, including Lipitor, is affected by side effects: patients taking these drugs must be monitored by their physician frequently to ascertain that toxic effects such as liver damage are not taking place.

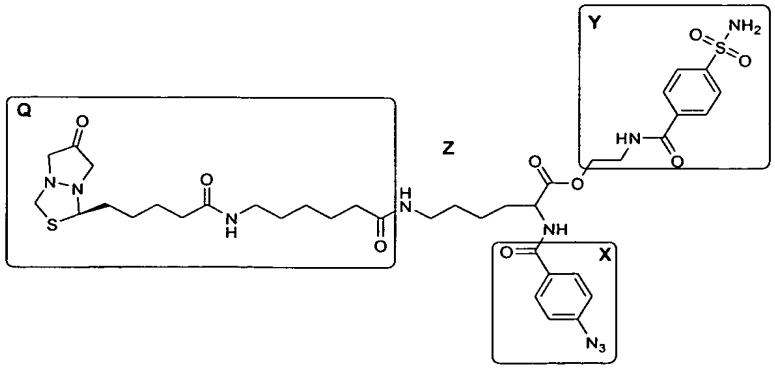
(3) Arthritis / Pain / Inflammation. Reports of gastrointestinal and in some cases coronary side effects have limited sales of the anti-inflammatory COX-2 inhibitors Vioxx and Celebrex, as many doctors recommend that their patients take safer but far less effective drugs such as ibuprofen to ease inflammation symptoms.

The description in the application, including the Figures, particularly Figure 30, which schematically depicts the method, and the working Examples describe how to practice the method and provides data showing practice each step of the method. As discussed below and in the previous response, all elements of the claimed method are described in the application: preparation of capture compounds is detailed and exemplified in Examples 1-13 as well as 14-16, as are reactions in which molecules are captured and analyzed. Examples 12 and 13 shows how to make exemplary capture compounds for use in the method claimed in this application, and Example 14 shows that how to use a capture compound that presents a drug to capture a target molecule in a sample. Example 16 is a working example in that it exemplifies all steps in the method as applied to assessing interactions of other drugs. The specification also teaches and exemplifies synthesis of capture compounds and provides working examples showing capture and analysis of captured compounds. Example 15

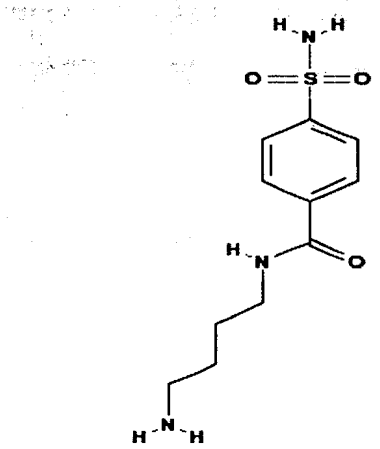
provides another use for these capture compounds. Figure 30 and Example 16 describe in detail how to apply such to the instantly claimed method steps. The specification includes Figure 30, which schematically depicts the method, and Example 16 which is a step-by-step example for identifying structural features that contribute to pharmacologic/therapeutic profile and differences in activity within an exemplary class of drugs, the thiazolidinediones and their metabolites. The overall process depicted in Figure 30 shows that a capture compound is mixed with a sample containing a mixture of proteins. Proteins with an affinity for the Y (e.g. drug) are allowed incubated with the sample to equilibrium with the Y function. The X moiety in the capture compound is then activated (for example, with electromagnetic radiation) forming a radical, which is short-lived, to covalently capture the proteins for which Y had an affinity. Proteins are not captured if the capture compound was not in very close proximity due to the equilibrium between Y function and such proteins. The capture compounds with captured protein are isolated, such is with biotin (Q), and can be identified using mass spectrometry or other identification method.

The results in Figures 31-38 demonstrate presenting a selected drug (i.e. an antibiotic) on a capture compound and detecting with what the drug interactions. Thus, all steps in the method are described and/or exemplified in the application.

The results in Figures 31-38 demonstrate the method as claimed. Figure 31 shows selective protein capture using capture compounds. Capture compounds A and B containing a sulfonamide (a drug) interact with Carbonic Anhydrase (CA). Capture compound A (and capture compound B), the elected species, is an example of a capture compound that presents a drug. In compound A on page 124, Q is biotin, X is an aryl azide, Y is an aryl sulfonamide **(a drug)** , and Z is lysine, as illustrated below:



Compound B that was prepared has a shorter biotin linker (Q). Aryl sulfonamides are known drugs. For example, capture compound A presents a 4-sulfamoyl benzene carboxamide. Exemplary of such is N-(4-aminobutyl)-4-sulfamoylbenzamide:



Sulfonamides, such as these, are known inhibitors of carbonic anhydrase, and are used as topical anti-glaucoma drugs (see, *e.g.*, Elbaum *et al.*, *J. Am. Chem. Soc.* 118:8381-87 (1996), which shows that the above sulfonamide binds to carbonic anhydrase II (the target); and Mincione *et al.*, *Bioorg. Med. Chem. Lett.* 11:1787-91 (2001), which shows that 4-sulfamoyl-benzene carboxamides (see, *e.g.*, page 1788, derivatives of compound 8, which is the above compound) have strong topical anti-glaucoma properties). Mincione *et al.* states, page 1787, in the introduction, that "[i]nhibition of carbonic anhydrase (CA, EC 4.1.1.1) with sulfonamides constitutes one of the most physiological approaches for the treatment of glaucoma." Further, column 1, page 1787 indicates that treatment is effected by inhibiting carbonic anhydrase II (CAII), which mediates bicarbonate secretion within the aqueous humor leading to elevated intraocular pressure. Thus, inhibition of CAII with sulfonamide drugs is a known treatment for CAII.

Working examples and Figures demonstrate use of the exemplary capture compounds that present this drug. The assays described in Example 14 were performed to determine the Kd. According to literature, the Kd of the sulfonamide for the CA II isoform is ~10nM, and for the CA I isoform is ~1uM. These values were independently confirmed using the assay as described in the Example, in which the capture compounds are incubated with a sample containing the CA isoform under conditions that allow it to reach equilibrium. The CA isoform is then captured using X. Using purified proteins, affinity and capture efficiency is highest for CA II, lower for CA I, and negligible for other purified proteins tested.

Figures 31-38 show practice of each step of the method: (1) contacting a capture compound with user-selected Y groups with a sample for a sufficient time for the interaction between the capture compounds and the biomolecules to reach equilibrium, (2) activating X to form a covalent linkage or high affinity bond with a biomolecule to effect capture thereof; and (3) identifying captured molecules is described and exemplified in the application.

Figure 32 shows relative binding strengths of protein isoforms to a known ligand for capture compound B. Figure 33 shows isolation of Carbonic Anhydrase from complex protein mixtures using capture compound A. CA II was doped into a FPLC purified protein mixture from the human kidney cell line HEK293. The doped CAII was pulled out from all other proteins using avidin-coated (SoftLink) resin. Other proteins were discarded, yielding purified protein ready for further analysis. Figure 33 shows incubation of the capture compound with a sample of purified proteins from a cell line that contains the target protein. The CAII was isolated and detected. Thus, Figure 33 shows practice of all steps of the method: (1) contacting a capture compound with user-selected Y groups with a sample for a sufficient time for the interaction between the capture compounds and the biomolecules to reach equilibrium, (2) activating X to form a covalent linkage or high affinity bond with a biomolecule to effect capture thereof; and (3) identifying captured molecules.

Figure 34 shows isolation of Carbonic Anhydrase from a highly complex protein mixtures using capture compound A. CA II was doped into the whole cytosolic extract from the human kidney cell line HEK293, . The doped CAII was pulled out from all other proteins using avidin-coated (SoftLink) resin. Other proteins were discarded, yielding purified protein ready for further analysis. Figure 34 also shows practice of all steps of the method.

Similarly Figures 35-38 show practice of all steps of the method. Figure 35 shows capture and isolation of Carbonic Anhydrase from lysed red blood cells. The top spectrum in the figure shows direct MALDI of lysed red blood cells (no purification) wherein signal for Hemoglobin, which is in huge excess over all other proteins, can be seen. Signals are seen for the alpha and beta chains, and also for non-specific dimers (~30 kiloDaltons). The bottom spectrum in the figure is taken after capture compound A, containing a sulfonamide drug with an affinity for Carbonic Anhydrase, is mixed with the lysed red blood cells. The capture compound covalently captures the Carbonic Anhydrase isoforms I and II. All other proteins that are not covalently captured, including nearly all of the Hemoglobin which is in 2-3 log excess, are washed away prior to MALDI analysis. No gel or chromatographic cleanup is required to obtain this spectrum. The intensity of the CA II peak is higher than CAI (which is

more ~100x more abundant in RBCs) because the sulfonamide drug has a higher affinity for CAII.

Figure 36 shows direct capture of Carbonic Anhydrase from red blood cells, without pre-lysis of the cells. Figure 37 shows capture of Carbonic Anhydrase from red blood cell lysate when unbiotinylated proteins including Carbonic Anhydrase are in huge excess. Figure 38 shows capture of proteins with lower affinities using very high concentrations of capture compound A.

Thus, these figures show that capture compounds that present drugs can be used to isolate proteins that interact with Y and for which Y has an affinity. In addition, Example 16 provides a step-by-step example for identifying structural features that contribute to pharmacologic/therapeutic profile and differences in activity within a structural subclass of drug, such as the thiazolidinediones, which are ligands of the PPAR-γ 2. PPAR-γ 2 predominantly is expressed in adipocytes, intestine, and macrophages and possibly muscle cells. Thiazolidinediones (Glitazones) include the drugs:Troglitazone (Rezulin™) Rosiglitazone (Avandia™) and Pioglitazone (Actos™). The anti-diabetic activity of thiazolidinediones is effected by binding to PPAR-γ (gamma) protein. Structure Activity Relationships (SARs) of thiazolidinediones and crystal structures of and PPAR-α co-crystallized with thiazolidinediones is known in the literature

The effect of thiazolidinediones on insulin sensitivity is mediated through altered expression of PPAR-γ 2- dependent genes. Thiazolidinediones, as anti-diabetic drugs, exhibit show toxicity and undesirable side effects. Thiazolidinediones (Glitazones): Troglitazone (Rezulin™) Rosiglitazone (Avandia™) and Pioglitazone (Actos™) are attached Y groups that are attached to Z moiety in the capture compound. These are designated CC-Thiazolidinediones. These are incubated with kidney, liver, pancreatic, colonic epithelium and muscle cells, which are cells in which PPAR-γ is expressed. Rezulin, Avandia and Actos will capture PPAR-γ, PPAR-α and also any non-target proteins with which each interacts. Since these three drugs have different metabolism and pharmacokinetics, they will capture different non-target proteins.

Since undesired and toxic side effects of each of the thiazolidinediones can be due to interaction with PPAR-α and non-target proteins, identification of the captured non-target protein for each drug provides insights into possible sources of side-effects. In addition, as described and claimed, the drugs then can be modified to eliminate these interactions. The modified drugs can be screened against kidney, liver, pancreatic, colonic epithelium and/or

muscle cells using these methods to confirm that the they are more specific for the PPAR-gamma target than the original drugs.

Example 16 describes this and also shows the structures of exemplary capture compounds (reproduced below) to be used in the method (incubation with kidney, liver, pancreatic, colonic epithelium and muscle cells for time sufficient to reach equilibrium, activation of the X group to capture the interacting molecules, and identification of the captured molecules). Example 16 clearly describes that the methods are for assessing the interactions of the presented drugs.

b. Declaration of Dr. Koster provides a study that demonstrates application of and the elegance and power of the claimed methods

The Declaration of Koster illustrates application of the claimed method and shows the power of the method in identifying targets and non-targets of a drug Y using capture compounds that present Y. The Declaration should render it clear how the method as claimed and practiced, is different from the prior art. Prior art methods that employ capture compounds (compounds with groups that covalently bind to reactive groups on proteins) seek to capture analytes of interest or to label molecules. The instant methods are designed to identify with what proteins a drug of interest interacts. Furthermore, the interacting portion of the capture compound Y is distinct from and separate from the portion of the molecule that captures the proteins that have an affinity for Y.

The study described in the Declaration shows this. This study has been published as well (see, Fischer *et al.* Toxicol Sci. 2010 Jan;113(1):243-53. Epub 2009 Sept 26). The study is described to help illustrate the power and elegance of the method claimed in this application. It shows that the methods can be used to identify a drug's non-targets with which it interacts *in vivo* and that such interactions can be responsible for undesirable side-effects. It also shows how the non-targets are identified and the causes of observed toxic side-effects are determined.

The Parkinson's drug tolcapone causes liver toxicity and hepatitis in patients, and at one time was removed from the market. Entacapone does not exhibit these side-effects. Both drugs are inhibitors of catechol-O-methyl transferase (COMT), which is the "target." For the experiments, the Parkinson's drugs Tolcapone and Entacapone (see structures in the Figures and schemes attached to the Declaration) were presented on capture compounds and tested in accord with the claimed methods. The capture compounds were tested with hepatocytes as tolcapone exhibits liver toxicity. The Declaration shows that an improved drug with fewer

side-effects interacts with fewer non-targets. It is shown that both drugs interact with the target, but the drug with toxic side-effects interacts with more non-target molecules. The identity of the non-target molecules explains the basis for the toxic side-effects.

As noted, the instantly claimed methods can be used for studying and assessing the interactions of drugs (or other such moieties, including enzymes) by presenting the drug (or fragment, metabolite, intermediate or prodrug thereof or an enzyme or substrate therefor) on a capture compound to assess the drug interactions and capture interacting molecules with an activatable moiety also presented on the compound. The capture compound can be a known capture compounds with an activatable group, X, that covalently binds to particular reactive groups on biomolecules, and a group, Q, for immobilization or separation. The capture compound is modified so that it also presents the drug, enzyme etc whose interactions are assessed. All of the experiments described in the Declaration were performed as claimed in this application.

In the study described in the Declaration, the cause of toxicity of a drug tolcapone for treatment of Parkinson's disease was determined using the instantly claimed methods by presenting the drug on a capture compound. The capture compound contained Z, an amino acid, Q, biotin for sorting, X a photoactivable group and presented the drugs whose interactions were assessed. In the study in the Declaration, capture compounds were prepared that present the drugs tolcapone or entacapone; hence each drug is a moiety "Y." Tolcapone and entacapone are potent inhibitors of catechol-O-methyl transferase (COMT; the target for each drug) and are for the treatment of Parkinson's disease. Tolcapone is a drug that has liver toxicity; and entacapone is an improved drug that is less toxic.

To assess their interactions, capture compounds were prepared as described in the application. In one capture compound, the drug tolcapone is presented; and in the other the drug entacapone is presented (see structures in the Figures and schemes attached to the Declaration). Each capture compound was incubated with cell lysates from hepatocytes to allow biomolecules in the lysate to come to equilibrium with the capture compound, the X groups were activated by exposure to light of the appropriate wavelength and the biomolecules in the lysate that interact with the drug (Y) were captured and then the capture compound-biomolecule complexes were separated from the mixture (via Q, the sorting function). The bound biomolecules were assessed by mass spectrometry. Both capture compounds bound to COMT, the target. The tolcapone-presenting capture compound (tolcapone is the drug with toxic side-effects), binds to significantly more biomolecules in

addition to COMT, than the capture compound that presents entacapone, which is the improved drug with fewer side-effects. Analysis of the captured biomolecules revealed that the tolcapone capture compound additionally binds to essential proteins in ß-oxidation pathways and oxidative phosphorylation pathways, from which the causes of the side-effects can be inferred. The experiments also demonstrate that, when presented on a capture compound, an improved form of the drug that does not exhibit the side-effects, and tested in accord with the claimed methods, does not interact with the drug non-targets. In this instance, not only were non-targets identified, but the results provided insights in the causes of the side-effects. The experiments and results are described in the Declaration of Koster and the attached figures and tables.

These experiments identify the non-targets with which a drug known to have deleterious side-effects (tolcapone) interacts and compares them with a redesigned version thereof (entacapone) that does not have the side-effects using the instantly claimed methods to assess the interactions. The experiments show that the instantly claimed methods indeed identify drug non-targets of the first compound, including those responsible for the side-effects. The experiments show that the redesigned drug does not interact with these non-targets. The experiments also show that identifying the drug non-targets can provide an understanding of the cause of the side-effects.

Capture Compounds

Capture compounds T1, T2 and T3 (see Figures in the Declaration) were synthesized in accord with the descriptions in the application using standard organic chemistry by linking Tolcapone to a capture that contains an aspartic acid Z moiety, an aryl azide reactivity function (X), and a biotin sorting function (Q). Capture compounds E1 and E2 (see Figures) were synthesized by attaching entacapone to the same scaffold capture compound as tolcapone. The hydroxy groups of tolcapone and entacapone were shown to be required for COMT (target) interaction. In compounds T1, T2 and T3 the 3-hydroxy of Tolcapone is linked to Z via a C2 linker, C4 linker, or C6 linker, respectively. In compound E1, the 3-hydroxy of Entacapone is linked to Z via a C2 linker. In compound E2, the N-ethyl group is directly linked to the capture compound. Thus, in compounds T1, T2, T3 and E1, the COMT-interacting moiety is occupied by the scaffold linkage. In compound E2, the COMT-interacting moiety is not occupied; thus, E2 also should interact with the target.

Capture Experiments with Human Hepatocytes

To perform the experiments, each capture compound was contacted with human hepatocyte lysates for a sufficient time for each presented drug to interact, and then the X moiety was activated with light to capture interacting compounds. In experiments with the human hepatocyte cell line HepG2 lysate, the capture compounds that present Tolcapone captured more proteins than the Entacapone capture compounds.

The results are shown in Figures 3-6 and Tables 1 and 2. Compound T1 captured 67 proteins that were not captured by E1 or E2, while compound E1 captured 10 proteins that were not captured by T1, T2 or T3. Overall, 20 proteins ("Entacapone hits") were captured by at least one Entacapone-presenting capture compound but not by a Tolcapone-presenting capture compound. In contrast, 124 proteins ("Tolcapone hits") were captured by at least one Tolcapone-presenting capture compound but not by an Entacapone-presenting capture compound.

The hit proteins were then analyzed by mass spectrometry to identify them. Tolcapone hits are proteins that occur in the Golgi apparatus, mitochondrion, endoplasmic reticulum, nucleus, cystosol, and peroxisome, and the majority occur are in membranes (Golgi apparatus, mitochondrion or endoplasmic reticulum). In contrast, the majority of the Entacapone hits occur in the nucleus. The Tolcapone hits include complexes in the Fatty Acid β-oxidation pathway and the respiratory chain (for example, ATP synthase, complex I, complex II and complex III), thus, confirming the source of the side-effects exhibited by Tolcapone, that are not exhibited by Entacapone (see slides 6 and 7).

Capture Experiments using Rat Liver

Mitochondrial proteins

In a further experiment, to confirm the above results, the capture compounds were incubated with rat liver mitochondrial proteins in a lysate. Experiments with rat liver mitochondria show that Entacapone-presenting compounds E1 and E2 captured fewer proteins than Tolcapone-presenting compounds T1 and T2 . Furthermore, compound E1, in which the COMT-interacting moiety is occupied, captured more non-target proteins than E2 in which the COMT-interacting moiety is presented. Compound E1 and E2 captured three proteins and one proteins, respectively, while compounds T1 and T2 captured nine and six proteins, respectively. Figure 3B depicts the components of the respiratory chain and shows that the Tolcapone-presenting compounds interact with members of this pathway, and also show which other mitochondrial proteins and members of the fatty acid beta-oxidation

pathway the compounds interact. These results show that Tolcapone interacts with quite a few non-target proteins, particularly mitochondrial proteins, thus, providing a mechanism for the observed toxicity.

Capturing drug non-targets (and target COMT) from the cytosolic fraction of rat liver

To illustrate how capture compounds can be used to identify drug moieties responsible for drug target and non-target interacting, capture experiments with the cytosolic fraction of rat liver cells were performed with compounds E1 and E2 (slide 10). SDS-PAGE of the captured proteins followed by silver staining identified only one protein band captured by compound E2. The band was the expected molecular weight of COMT (target) and was confirmed as COMT by western blotting and mass spectrometry. In contrast, compound E1 captured three other proteins (peroxisomal multifunctional enzyme type 2, P97852; peroxisomal acyl-coenzyme A oxidase 3, Q63448; and methylglutaconyl-CoA hydratase Q9JL23), but not COMT, since its site is occupied. No bands were observed in negative control reactions with compounds without a drug moiety (Slides 11-13 show the results and protein identification by mass spectrometry).

Conclusion

As described in the Declaration, the experiments in the study illustrate that the claimed methods using capture compounds that present a drug (drug fragment, metabolite, intermediate or prodrug) and an activatable moiety X that covalently captures biomolecules, can be used to identify drug targets **and** non-targets just as described and claimed in the application. When these capture compounds are used in the instantly claimed methods in which the capture compounds are contacted with a sample for a sufficient time for all interactions to reach equilibrium and then an activatable group X is activated to covalently catch the interacting molecules, interacting drug non-targets are captured. Analysis of drug non-targets can provide insights into causes of side-effects and drugs can be redesigned to reduce such effects. The Tolcapone-presenting compounds captured proteins involved in the respiratory pathway in liver mitochondria. These proteins were not captured by Entacapone-presenting compounds. Tolcapone exhibits liver toxicity; whereas Entacapone does not. Hence, not only did the methods permit identification of drug non-targets with which the drug Tolcapone interacts, but also provided an understanding of the cause of the toxicity.

In addition to identifying drug non-targets of different drugs, the methods herein using capture compounds that present drugs, can identify which moieties in a drug are

responsible for various interactions. Compound E2 in which the COMT interacting site is exposed, captures COMT, the target. In contrast, compound E1 in which the COMT-interacting site is blocked does not capture COMT.

Therefore, by identifying drug targets and non-targets, the instantly claimed methods can be used, for example, for risk assessment and safety evaluation of candidate drugs as well as to redesign design drugs to reduce interactions with non-targets. The claimed methods can facilitate the optimization of safer drugs with decreased side effects and reduce the attrition rate in drug discovery.

The above discussion and Declaration should aid in appreciating the claimed methods. This discussion is referenced in the discussion below as appropriate.

THE REJECTION OF CLAIMS 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158161, 163, 164, 166 and 169 UNDER 35 U.S.C. §112, SECOND PARAGRAPH

Claims 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158161, 163, 164, 166 and 169 are rejected under 35 U. S. C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention for reasons discussed in turn below. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and/or the following remarks.

Relevant law

The purpose of 35 U.S.C. §112, second paragraph, is to provide those who would endeavor, in future enterprise, to approach the area circumscribed by the claims of a patent, with adequate notice demanded by due process of law, so that they may readily and accurately determine the boundaries of protection involved, evaluate the possibility of infringement and dominance by determining the metes and bound of protection so one can evaluate the possibility of infringement with a reasonable degree of certainty. *In re Hammack,* 427 F.2d 1378, 166 USPQ 204 (CCPA 1970). Claims are not to be read in a vacuum, and the limitations therein are to be interpreted in light of the specification, giving them their broadest reasonable interpretation. When one skilled in the art would understand all of the language in the claims when read in light of the specification, a claim is not indefinite. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983).

Claims are not read in a vacuum but instead are considered in light of the specification and the general understanding of the skilled artisan. *Rosemount Inc. v. Beckman Instruments, Inc.*, 727 F.2d 1540, 1547, 221 USPQ 1, 7 (Fed. Cir. 1984), *Caterpillar Tractor Co. v. Berco, S.P.A.*, 714 F.2d 1110, 1116, 219 USPQ 185, 188 (Fed. Cir. 1983). Claim language is satisfactory if it reasonably apprises those of skill in the art of the bounds of the claimed invention and is as precise as the subject matter permits. *Shatterproof Glass Corp. v. Libby-Owens Ford Col.*, 758 F.2d 613, 624, 225 USPQ 634, 641 (Fed. Cir.), cert. dismissed, 106 S.Ct. 340 (1985).

Analysis

1) **Claim 1** The Examiner states that Claim 1 recites:

> identifying targets and non-targets of a drug" and "wherein the captured biomolecules comprise drug targets and non-targets", which recitations are unclear and render the said claim indefinite. The instant claims seem to recite conflicting limitations. It is not clear how a compound/molecule can be both a drug target as well as a non-target simultaneously. The instant claim 1 seems to recite due both of the "drug target" and the "non-targets" are identified by binding or bonding to the capture compound. Thus, it is not clear how a single function of "binding" can lead to opposite results of a target and a non-target.

As discussed above, in great detail, the method is for identifying all proteins (as presently claimed) in a sample that interact with the drug (Y). Proteins that interact with a drug will include target proteins, but also, will include other proteins, designated in this application as non-targets (i.e. proteins that are not the target of the drug). These are not conflicting limitations, but describe the results of the method. The method as practiced identifies molecules that interact with Y; they are captured by activating X, which captures whatever is interacting with Y. This is why and how the instantly claimed methods differ from the prior art, which use capture compounds and methods that are designed to capture targeted analytes. Typically a prior capture compound has a moiety that covalently binds to the target; **and it is the interactions of the covalently binding moiety that is assessed**. In the instantly claimed methods, the interacting moiety whose interactions are assessed is **not** the covalently binding moiety. The covalently binding moiety is going to capture anything in its vicinity (i.e. in the crude analogy discussed above Y is the nectar with which the good bugs (targets) and bad bugs (non-targets) interact and X is venus fly trap that upon activation slams shut and captures the good and bad bugs). As a result, the instantly claimed methods and the capture compounds employed therein capture targets and non-targets of Y, the drug.

2) Claims 1 and 2 The Examiner continues:

> Claims 1 and 2 recite the term "non-targets" or drug non-targets, which term seems to be in conflict with the method steps of the instant claim 1. The instant specification defines the term "drug non-target" as "a biomolecule, such as a protein including but not limited to receptors and enzymes, that the drug is not intended to interact with in vivo." However, the instant claimed method requires the biomolecules "interact" (bind) with the capture molecule through the "drug" (i.e. "biomelecule(s) in the sample that interact with Y"). Thus, the term "drug non-target" conflicts with the required method step of "interact" with the drug.

Based on the discussions above, it should be clear that Y, the drug, interacts with its targets and non-targets, and X, when activated captures whatever is interacting with Y. X does not specifically bind, but captures proteins. The capture interaction is not specific to targets or non-targets, X is going to capture whatever is in the vicinity. The methods assesses the interactions of Y. Y interacts with its targets, and, can interact, as most drugs seem to, with non-targets.

3) Claim 163

Claim 163 is rejected in the recitation of "the mass spectrometry format" in lines 1 and 2 because there allegedly is insufficient antecedent basis for this limitation in the claim. Reconsideration of the grounds for this rejection is respectfully requested in view of the amendments herein and the following remarks.

As amended claim 163 recites:

> The method of claim 110, wherein ~~the~~ mass spectrometric analysis is performed with a mass spectrometric analysis format that is selected from among matrix assisted laser desorption ionization (MALDI), continuous or pulsed electrospray (ES) ionization, ionspray, thermospray, and massive cluster impact mass spectrometry.

Claim 110 recites "The method of claim 1, further comprising identifying or detecting a captured biomolecule by **mass spectrometric analysis**". Claim 110, thus provides exact antecedent for "mass spectrometric analysis.

Claim 163 also recites that the mass spectrometric analysis is performed with a mass **spectrometric analysis format** that is selected from among . . . . Claim 163, thus, refers to the antecedent and then states with what the analysis is performed (i.e. the format). Claim 164 refers to the mass spectrometric analysis format . . . , thus referring to its exact antecedent in claim 163:

164. (Currently Amended) The method of claim 163, wherein **the mass spectrometric analysis** ~~detection~~ **format** is linear time-of-flight (TOF), reflectron time-of-flight, single quadruple, multiple quadruple, single magnetic sector, multiple magnetic sector, Fourier transform, ion cyclotron resonance (ICR), or ion

THE REJECTION OF CLAIMS 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158- 161, 163, 164, 166 and 169- New matter

Claims 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158-161, 163, 164, 166 and 169 are rejected under 35 U.S.C. 112, first paragraph, as containing new matter. It is alleged that claim 1 is amended to recite that the "specification and claims as originally filed do not disclose methods where both "targets and non-targets of a drug" are identified together through the covalent binding to the capture compound." This rejection is respectfully traversed.

First, it should be apparent from the discussion above, that the method by the way it is practiced identifies targets and non-targets of the presented small molecule "Y." As described above, the capture compound that presents Y is incubated with a sample such that all proteins, which include targets of Y and non-targets, in the sample that interact with Y interact with Y. X, which, upon activation, covalently binds to proteins, captures all proteins that are interacting with Y, which include targets and non-targets. This is the result of the method (see *e.g.*, Figure 30, which depicts the method and shows molecules interacting with Y by virtue of an affinity therefor, and then activation of X to covalently capture molecules in the vicinity of Y).

Second, the application as originally filed describe that methods for identifying targets and non-targets of Y. For example, page 6, lines 4-28 recite:

> The capture compounds, collections and methods provided herein also permit screening of biomolecules, including but not limited to receptor proteins and enzymes, **which are drug targets and non-targets**, as defined herein, that interact with pharmaceutical drugs under physiological conditions. The screening of biomolecules provides increased understanding of the mechanism of action of the pharmaceutical drugs or drug fragments, metabolites or synthetic intermediates in the drug syntheses, thereby helping the design of more target specific drugs. The methods also provide for identification of non-target biomolecules, such as proteins including but not limited to receptors and enzymes, that interact with pharmaceutical drugs, thereby causing side effects and other undesired therapeutic effects. In one embodiment, various attachments of the drugs or drug fragments, metabolites or synthetic intermediates in the drug syntheses to the capture compounds are used to determine which functionalities of the drugs or drug fragments, metabolites or synthetic intermediates in the drug syntheses interact with the target and non-target biomolecules. In one embodiment, the non-target functionalities are then eliminated from the drug, resulting in an improved drug that exhibits fewer side effects. In another embodiment, a drug is included in the capture compound, proteins that interact with the drug are isolated and identified,

> the proteins are related to function, and the drug is re-engineered to eliminate or reduce interactions with non-target proteins. The method may be repeated on the re-engineered drug, as desired.

At page 160, lines 14-21, the specification recites:

> In one embodiment, the capture compounds/collections thereof are designed to contain pharmaceutical drugs/drug fragments, drug metabolites or prodrugs as the selectivity function and suitable reactivity and sorting functionality. In the methods provided herein, the capture compound/collections thereof are allowed to interact with **a mixture of drug target and non-target biomolecules, including but not limited to, receptor proteins. The captured biomolecules are then analyzed to identify drug target and non-target biomolecules.**

At page 161, line 25, page 162, line 2, the specification recites:

> Following the procedures described above, the proteins (target and non-targets) that interact with the drug and their respective putative function are identified, screening against all cell types potentially involved in the therapeutic or side-effect-related pathways. Knowledge of the therapeutic effect of the drug, as well as its side effects as previously observed in patients, facilitate the formation of a hypothesis as to which of the captured proteins lead to the desired therapeutic effect, and which are involved in its side effects

Therefore, amendment of the claim to recite that the method is for assessing the interactions of drug, or fragment, metabolite, intermediate or prodrug thereof, does not add new matter.

In addition, while absolutely not conceding that this rejection has merit, which as discussed above, it does not, in the interest in advancing claims to allowance, they are amended to delete explicit reference to drug targets. The claimed methods, however, do, as discussed extensively herein, capture targets and non-targets of the small molecule Y, because the methods are designed to capture all proteins that interact with Y.

THE REJECTION OF CLAIMS 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158-161, 163, 164, 166 and 169 AND 169 UNDER 35 U.S.C. §112, FIRST PARAGRAPH- SCOPE OF ENABLEMENT

Claims 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158-161, 163, 164, 166 and 169 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a limited number of X, Y, Q and Z substituents like biotin, small molecular weight drugs of known composition, a select number of known "latent" photoactivatable groups like azides, does not reasonably provide enablement for the use of "any" X, Y, Q and Z. The Examiner states that the specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. This rejection respectfully is traversed. The Examiner's comments are discussed in turn below.

It respectfully is submitted that some or all of claims 2, 6, 10, 43, 44, 46, 137-139, 163, 164 and 175 are outside the purview of this rejection. These claims recite specific groups for X, Z and Q that the Examiner states are enabled. Regarding "Y," as discussed throughout this response, Y is a user selected small molecule drug (or a fragment, metabolite, intermediate or prodrug thereof that interacts with a non-target of the drug). The purpose of the method is to identify non-target proteins that interact with a drug of interest. The user selects a drug and presents it on a capture compound and practices the steps of the method to isolate or identify from a sample protein non-targets with which the drug interacts. It is of no value to limit a method for assessing the interactions of a drugs (or any small molecules) to particular drugs, since one of skill in the art will practice it with any drug of interest, not just the drugs discussed in the specification to exemplify practice of the method. It makes no more sense to so-limit the claims than to limit a method of drug discovery to particular drugs, since such drugs are already discovered. The method is designed to be practiced on drugs for whom one of skill in the art wishes to identify non-targets.

The Examiner is reminded that the claims are directed to methods, not to compounds. The claims do not encompass an infinite number of methods; the claims clearly recite the steps of the method: selecting a drug whose interactions are of interest and providing it on a capture compound that includes a group Q for sorting/immobilizing and a photoactivatable group X that covalently binds to proteins presented on a trifunctional core Z; contacting the capture compound that presents the drug (or fragment, intermediate, metabolite or prodrug thereof) with a sample for a time sufficient to reach equilibrium with Y so that proteins in the sample that have affinity for Y will be in the vicinity of Y; activating X to covalently capture the proteins with which Y interacts; and identifying isolating the captured proteins to identify non-targets of the drug. These are steps of the method; the user selects the drug of interest and presents it on a capture compound.

Relevant Law

Enablement is a legal determination that assesses whether a specification teaches one of skill in the art to make and use what is claimed. Enablement is not precluded even if some experimentation is necessary, as long as the amount of experimentation is not undue. *Atlas Powder Co. v. E. I. Du Pont De Nemours Co.,* 750 224 USPQ 409, 3 (Fed. Cir. 1984); *W. L. Gore and Associates v. Inc.*, 721 220 USPQ 303, 315 (Fed. Cir. 1983).

The test of enablement is whether one skilled in the art can make and use what is claimed based upon the disclosure in the application and information known to those of skill in the art without undue experimentation. *United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988). A certain amount of experimentation is permissible, as long as it is not undue. To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. *Atlas Powder Co. v. E.I. DuPont de Nemours*, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be satisfied by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything **within the scope** of a broad claim." *In re Anderson*, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original.

The "invention" referred to in the enablement requirement of section 112 is the claimed subject matter. *Lindemann Maschinen-fabrik v. American Hoist and Derrick Co.*, 730 F.2d 1452, 1463, 221 USPQ 481, 489 (Fed. Cir. 1984) ("The question is whether the disclosure is sufficient to enable those skilled in the art to practice the claimed invention"); *Raytheon Co. v. Roper Corp.*, 724 F.2d 951, 956, 220 USPQ 592, 596 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 835, 225 USPQ 232 (1984).

> As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support. Assuming that sufficient reason for such doubt does exist, a rejection for failure to teach how to make and/or use will be proper on that basis; such a rejection can be overcome by suitable proofs indicating that the teaching contained in the specification is truly enabling. . . it is incumbent upon the Patent Office, whenever a rejection on this basis is made, to explain why it doubts the truth or accuracy of any statement in a supporting disclosure and to back up assertions of its own with evidence or reasoning which is inconsistent with the contested statement.

Id. (emphasis in original); *See also Fiers v. Revel*, 984 F.2d 1164, 1171-72, 25 USPQ2d 1601, 1607 (Fed. Cir. 1993);, *Gould v. Mossinghoff*, 229 USPQ 1, 13 (D.D.C. 1985), *aff'd in part, vacated in part, and remanded sub nom. Gould v. Quigg*, 822 F.2d 1074, 3 USPQ2d 1302. A patent application need not teach, and preferably omits, what is well known in the art. *Spectra-Physics, Inc. v. Coherent , Inc.*, 3 USPQ2d 1737 (Fed. Cir. 1987).

The starting point in an evaluation of whether the enablement requirement is satisfied is an analysis of each claim to determine its scope. The focus of the inquiry is whether everything within the scope of the claim is enabled. As concerns the breadth of a claim relevant to enablement, the only relevant concern should be whether the scope of enablement provided to one skilled in the art by the disclosure is commensurate with the scope of protection sought by the claims. *In re Moore*, 439 F.2d 1232, 169 USPQ 236 (CCPA 1971). Once the scope of the claims is addressed, a determination must be made as to whether one skilled in the art is enabled to make and use the entire scope of the claimed invention without undue experimentation.

The inquiry with respect to scope of enablement under 35 U.S.C. § 112, first paragraph, is whether it would require undue experimentation to make and use the subject matter as claimed. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); *see also In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

The requirements of 35 USC §112, first paragraph, can be fulfilled by the use of illustrative examples or by broad terminology. *In re Anderson*, 176 USPQ 331, 333 (CCPA 1973):

> ... we do not regard section 112, first paragraph, as requiring a specific example of everything within the scope of a broad claim .... What the Patent Office is here apparently attempting is to limit all claims to the specific examples, not withstanding the disclosure of a broader invention. This it may not do.

In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960) :

> It is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species. It is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it.

Analysis

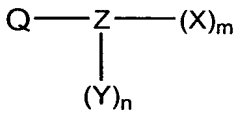
To establish undue experimentation, a consideration of the "*Wands* factors" as a whole is warranted. In this instance, as discussed below, an assessment of the *Wands* factors, which include the nature of what is claimed, the breadth of the claims, the teachings in the specification, the quantitation of experimentation necessary, the guidance provided by the

specification, the presence of working examples, the state of the art, the knowledge of those of skill in the art and the level of predictability in the art, leads to the conclusion that one of skill in the art can practice the methods as claimed without undue, experimentation.

a. The breadth of the claims and nature of the claimed subject matter

Claim 1 is directed to a method for identifying targets and non-targets of a drug by:

> (a) selecting a small organic molecule drug whose non-targets with which it interacts are to be identified, and providing a capture compound that presents the drug or a fragment, intermediate, metabolite or prodrug of the drug whose non-targets are to be identified , wherein the fragment, intermediate, metabolite or prodrug of the drug interacts with a non-target of the drug;
>
> (b) contacting the capture compound with a sample containing non-target proteins that interact with Y, wherein contacting is effected under conditions in which X is not activated and for a sufficient time for interaction between the capture compounds and proteins in the sample to reach equilibrium, whereby Y interacts with drug non-target proteins in the sample;
>
> (c) exposing the capture compound to electromagnetic radiation that activates X, whereby X forms a covalent linkage with protein(s) in the sample that are interacting with Y to effect capture thereof; and
>
> (d) determining the identity of captured proteins, wherein the captured identified proteins comprise non-targets of the drug.
>
> The capture compounds are of formula:



in which

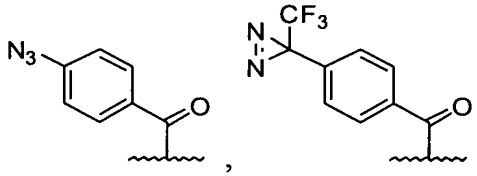
> m is 1;
>
> n is 1;
>
> X is a photoactivatable group that, upon exposure to light, covalently binds to an amino acid side chain of a protein to effect covalent binding of the capture compound to a protein;
>
> Y is the small molecule organic drug or a fragment, intermediate, metabolite or prodrug thereof for assessing interactions with non-targets;
>
> Q is a sorting function for immobilizing or separating the capture compounds; and
>
> Z is a trifunctional group containing 50 or fewer atoms that presents each of X, Y and Q.

Dependent claims specify particulars regarding Z, Y, X and Q as well as the method, including additional steps, such as redesign of the drug to reduce interaction with drug non-targets.

In particular, claim 2 recites the method of claim 1 where Z comprises an amino acid; and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid (PNA) and an activated dextran (exactly as recited in the specification at pages 122-123 (section 4, which describes Q).

Claim 6 further defines X, Z and Q: X is an azide or a diazarine; Z is an amino acid; and Q is biotin or an oligonucleotide. Thus in claim 6, X, Z and Q are specifically defined. As noted, Y is user selected, small molecule pharmaceutical drug or drug fragment, drug intermediate, drug metabolite or prodrug thereof whose interactions are assessed. Thus, claim 6 should not be within the purview of this rejection.

Claim 43, recites the method of claim 34, which defines Z as (S1)tM(R15)a(S2)bL, and states that M is an amino acid; S^1 and S^2 each is independently $(CH_2)_r$, where r is 1-10; and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid and an activated dextran.

Claim 44 is outside the purview of this rejection. Claim 44, which is dependent on claim 1, states that X is a diazirine, 3-trifluoromethyldiazirine or an azide; Z is an amino acid and Q is biotin. Claim 175 recites that X is 

or an arylazide; Z is selected from serine, threonine, lysine, tyrosine and cysteine; and Q is biotin or an oligonucleotide.

Claims 137, 139 and 140 recite that Z is (S1)tM(R15)a(S2)b, the components are as defined in claim 43. Claim 139 recites that M is an amino acid, and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid and an activated dextran; and claim 140 recites that X is an azide, S1 and S2 each is independently (CH2)r, where r is 1-10, and Q is biotin or an oligonucleotide. Thus, at the very least claims 6, 44, 140 and 175

cannot be within the purview of the rejection because all components of the capture compound are defined and recited.

As described in the application and claimed, the methods claimed in this application are methods for identifying molecules in a sample with which a drug, drug fragment, drug metabolite and/or prodrug interacts. Such molecules will include the targets for the drug and also any so-called non-targets with which a drug may interact. Non-targets can be responsible for side-effects or lack of specificity of particular drugs. Identification of such non-targets can aid in understanding a drug's activity and/or aid in redesign to eliminate such interactions. Identification of targets can be of interest for drugs whose targets are not known.

Thus, in practicing the method, the capture compound or compounds are contacted with a sample, such as a cell or tissue sample, under conditions such that the interactions with Y reach equilibrium but X is not activated. X is then exposed to electromagnetic radiation, which activates it so that it covalently binds to anything in its vicinity that has amino acid side chains. This includes all proteins that are interacting with Y. The captured biomolecules can then be identified. For example, if Y is a drug, a drug typically has a target. When contacting the compound(s) with a cell or tissue sample, Y should interact with its target. In addition, in this method, the capture compounds also will capture non-targets with which Y interacts. Identification of such non-targets can be used to predict side-effects and to aid in design of drugs that have reduced interaction with non-targets, and, hence fewer side-effects. The method can be used to assess affinities (*i.e.*, Kd of a drug, drug fragment, drug intermediate, drug metabolite or prodrug with its target and/or non-target.

The capture compounds used in the methods as claimed also ca include a Q moiety for sorting compounds, such as for capturing them on a solid support, and optionally include a W moiety that can alter solubility properties of captured compounds so that they can interact with biomolecules in a sample in various environments, such a hydrophobic environment that exists in a cell membrane, or a hydrophilic environment. Hence the instant methods can probe selected environments depending upon properties of the capture compounds conferred by W.

As discussed above, the methods assess the interaction of biomolecules in a sample with a moiety, Y, which is a small molecule drug or fragment, intermediate, metabolite or prodrug thereof whose interactions with non-target proteins are of interest to the user. **Hence Y is selected by the user.** Small molecule drug compounds are well known in the chemical

and medical arts (*e.g.*, the see *Small Molecule - Protein Interactions* (Waldmann & Kpooitz, eds., Springer-Verlag, Berlin; (2003), referencing work well before 2003); and Cho *et al.*, *Macromolecular versus small-molecule therapeutics: drug discovery, development and clinical considerations*, TIB TECH 14: 153-158 (1996)). Small molecule drugs were known in the art at the effective filing date of the instant application and readily could be selected by the skilled artisan, such as by consulting any formulary known in the pharmaceutical arts, such as *The Physicians Desk Reference* (46th edition, 1992), *Goodman & Gilman's The Pharmacological Basis of Therapeutics* (9th edition, 1996), *Remington's Pharmaceutical Sciences* (17th edition, 1985), and *Martindale: The Extra Pharmacopoeia* (31st edition, The Pharmaceutical Press, London (1996)), or the relevant editions of the United States Pharmacopeia (USP), British National Formulary, the British Pharmacopoeia or the European Pharmacopoeia. In addition, the skilled artisan who wishes to identify non-targets of a particular drug, by definition, knows the drug to present on a capture compound.

The method requires that the interaction of the protein with the capture compounds is performed under conditions such that the Y moieties reach equilibrium with proteins in a sample that contains non-target proteins under conditions in which X is not activated; then covalent binding is effected between any such proteins and X by activating X, the reactivity function, to capture the proteins that interact with Y.

Z is any core for presenting X, Q and Y. For practicing the method, Q, X and Y, as well as Z, can be selected as described in the application. Selection of Y depends upon the molecule(s) whose interactions is/are selected for assessment. Hence the practitioner of the method selects Y. X is a photoactivatable moiety that, when activated, captures the proteins that interact with Y. The claim specifies that biomolecules are capture by X either by covalent linkage. The specification provides detailed and extensive description of Z moieties, and X moieties as well as examples Y moieties.

The claims are not directed to compounds, but are directed to methods. The methods involve employing compounds with two functional moieties – one that is selected by a user to be assessed, and a second that forms covalent attachment or high affinity linkage to a biomolecule, such as a protein. As discussed in great detail above and below, the application provides examples of practice of the method as claimed.

The Examiner states:

> The breadth of the claims and the nature of the invention: Applicant's claims are directed to a broad genus of methods for isolating and identifying biomolecules that have been "captured" by a capture compound of formula Q-Z-(YA)$_n$/m. The Q moiety is described as a sorting function. Y is a pharmaceutical drug, drug fragment, drug intermediate, drug metabolite or prodrug. X is a ligand to a biomolecule that binds with sufficiently high affinity so that it will be "stable" under mass spectrometric analysis. And Z is moiety for presenting X, Y and Q. Thus, the claims encompass virtually an infinite number of methods employing virtually an infinite number of capture compounds because no structural limitations have been set forth. That is, Applicants have not limited the number of atoms, types of atoms, or the manner is way said atoms can be connected in defining the Q, X, Y and Z moieties. They could be composed of any element in the periodic table. Furthermore, the dependent claims also fail to limit at least one of the X, Y, Z, and Q moieties to anything less than an infinite number of possibilities. Thus, Applicant's claims encompass the entire universe of drugs, drug fragments, drug metabolites, sorting functions, ligands, etc. without exception. Consequently, the nature of the invention cannot be fully determined because the invention has not been defined with particularity.

As discussed above, the claims set for the steps of the method and recite that a user selected drug (or a fragment, metabolite, prodrug or intermediate thereof) is presented on a capture compound. The structure of the compound is set forth in the claims, and X, Z and Q are described in the claims (and as discussed below are well-known to those of skill in the art and exemplary groups are described in the specification). The compounds cannot be composed of any elements in the periodic table, the compounds used in the methods are capture compounds that, as amended, covalently capture proteins. The drugs for Y are user selected. Those of skill in the art are very familiar with small molecule drugs.

The claims are directed to the methods, not to the compounds. It is not relevant that a variety of capture compounds can be employed; it is only relevant that, in light of the specification and other factors discussed below, one of skill in the art can practice the steps of the method to identify non-target proteins of a small molecule drug of interest.

b. The level of skill in the art and knowledge of those of skill

Applicant respectfully submits that the skill in the art of chemical synthesis is high, and is so-recognized by the Examiner. Similarly, the knowledge of those of skill in the art is extensive. This is evidenced by the art in this area, which is authored primarily by those with Ph.D. and M.D. degrees and is intended for an audience of similarly highly skilled individuals, primarily in the fields of biochemical, pharmaceutical, or medical arts. The numerous articles and patents made of record in this application, authored and reviewed by those known in the art, address a highly skilled audience, and further evidence the high level

of skill in this art. Synthetic schemes describing the synthesis of the disclosed compounds are provided in the specification.

c. The state of the prior art

There is extensive prior art in which capture compounds that present X moieties are used to capture biomolecules in samples. Such art is of record in this application. See, *e.g.*, , Hutchens *et al.* (WO 98/59360), Cravatt *et al.* (WO 01/77668 and WO 01/77684), and Coull *et al.* (EP 0424 819), which are of record in this application. The capture compounds described in such publications, while not described for use in the instantly claimed methods, nor presenting a "Y moiety," as required by the instant claims, include moieties, designated X herein, for covalent capture of biomolecules. They also contain a core "Z" that presents X. Hence, those of skill in the art are well-acquainted with Z moieties and X moieties suitable for use in the compounds for use in the instantly claimed methods.

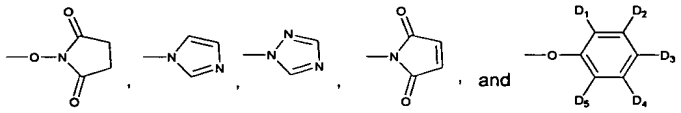
Attached hereto is a spreadsheet summarizing exemplary prior art publications that describe Q, X and/or Z moieties and/or capture compounds that can be to prepare capture compounds on which Y can be presented for use in the method.

Further, as stated above, Y is user selected drug or a fragment, metabolite, intermediate or prodrug thereof that interacts with non-target with which the drug interacts. The skilled artisan desirous of identifying non-targets of a particular drug, to, for example assess side-effects, would know the identity of the drug of interest. As noted above, small molecule drug compounds are well known in the chemical and medical arts (e.g., see *Small Molecule - Protein Interactions* (Waldmann & Kpooitz, eds., Springer-Verlag, Berlin; (2003)); and Cho *et al.*, *Macromolecular versus small-molecule therapeutics: drug discovery, development and clinical considerations*, TIB TECH 14: 153-158 (1996)). Small molecule drugs were known in the art at the effective filing date of the instant application and readily could be selected by the skilled artisan, such as by consulting any formulary known in the pharmaceutical arts, such as The Physicians Desk Reference (46th edition, 1992), Goodman & Gilman's The Pharmacological Basis of Therapeutics (9th edition, 1996), Remington's Pharmaceutical Sciences (17th edition, 1985), and Martindale: The Extra Pharmacopoeia (31st edition, The Pharmaceutical Press, London (1996)), or the relevant editions of the United States Pharmacopeia (USP), British National Formulary, the British Pharmacopoeia or the European Pharmacopoeia.

Q is for sorting/immobilizing/detecting compounds. The above-noted exemplary publications, as well as describe such groups. Methods in which compounds are immobilized are well-known.

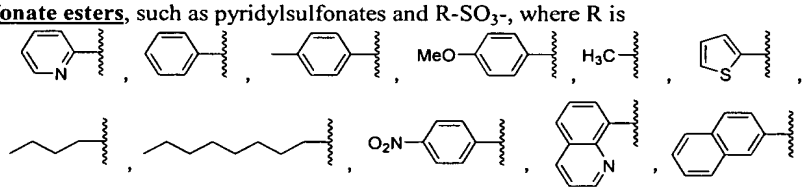
A brief review of art of record in this application that describes compounds with X, Q and/or Z moieties shows that those of skill in the art are familiar with each such moiety, including photoactivatable X moieties. Among this art, is included capture compounds that could be used in the claimed methods to present Y. The art and identity of some Z, Q and X groups in each reference summarized in the TABLE below. X groups that are not photoactivatable are included, as some of the references describe capture compounds with such X groups. These references are included for their teachings regarding Z and Q groups; none disclosure compounds that include all limitations of the compounds used in the method, but each disclose capture compounds or X, Z and Q moieties that can be combined as described in the instant application, or capture compounds that could be modified by including a Y group as claimed herein. Taken as a whole, these references show that at or before the earliest claimed priority date, those of skill in the at could prepare capture compounds with X, Z and Q groups and then modify them to present a drug (or fragment, intermediate, metabolite or prodrug) thereof.

Table: Examples of moieties Z, capture moieties X, and sorting functions Q. (Photoactivatable moieties X are indicated)

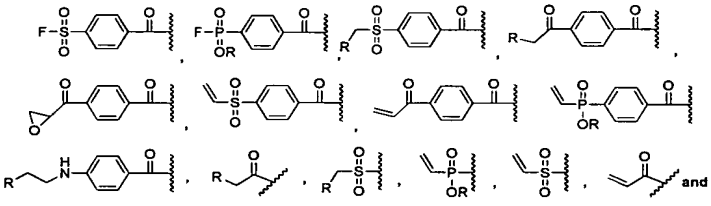
Document	Moiety	Example(s)
US 5,532,379	**X**	**Phenylazide (photoactivatable)**; and **N-hydroxysuccinimidyl esters.**
	Z	**-CH**
	Q	**Biotin**
US 5,410,068 and EP 0 424 819	**X**	**Active esters**, such as: **1)** N-hydroxysuccinimidyl ester; **2)** 2-nitrophenyl ester; **3)** 4-nitrophenyl ester; **4)** 2,4-dichlorophenyl ester; **Other Moieties**, such as acyl halides, acyl azolides, alkyl halides, sulfonyl halides or any reactive halide derivative; and $-(CH_2)_nC(O)W$, $-(CH_2)nSO_2W$, $-(CH_2)_nW$, where *n* is an integer from 0 to 20; and W is selected from the group consisting of Cl, Br, I, -NCS, -NCO,  where D1-D5 are the same or different and selected from H, F, Cl, Br, I, -NO2 and -CN.
	Z	**Substituted trityl groups**, such as 4,4'-dimethoxytrityl
	Q	**n/a**

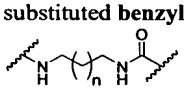
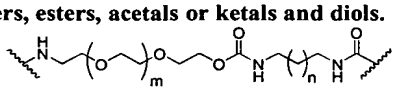
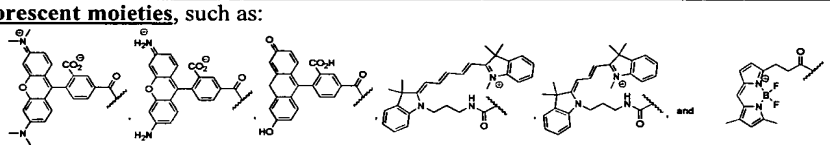
Document	Moiety	Example(s)
US 6,670,194 (first published in WO2000011208A1)	X	**Peptide Reactive Groups**, such as: 1) **Thiol reactive groups**, such as epoxides, α-haloacyl groups, nitriles, nitriles, sulfonated alkyl or aryl thiols and maleimides; 2) **Amino reactive groups**, such as sulfonyl halides, isocyanates, isothiocyanantes, active esters (including tetrafluorophenyl esters and N-hydroxysuccinimidyl esters), acid halides, acid anyhydrides, aldehydes or ketones in the presence or absence of $NaBH_4$ or $NaCNBH_3$, α-haloacyl groups, iodoacetylamides, and pentafluorophenyl esters; 3) **Carboxylic acid reactive groups**, such as amines or alcohols (in the presence of a coupling agent, such as dicyclohexylcarbodiimide, or 2,3,5,6-tetrafluorophenyl trifluoroacetate and in the presence or absence of a coupling catalyst such as 4-dimethylaminopyridine; and transition metal-diamine complexes, including Cu(II)phenanthroline.) 4) **Other peptide reactive groups**, such as groups that react with sulfydryl, homoserine lactone groups); carboxylate groups, and ester groups; **Ester reactive groups**, such as amines which react with homoserine lactone; **Phosphate reactive groups**, such as chelated metals, for example Fe(III) or Ga(III), chelated to, for example, nitrilotriacetiac acid or iminodiacetic acid; **Aldehyde or ketone reactive groups**, such as amines treated with $NaBH_4$ or $NaCNBH_3$ that can react with carbohydrates treated with periodate; and **Enzyme Substrates:** such as 1) substrates for an enzyme associated with a disease state or birth defect or one that is routinely assayed for medical purposes; 2) substrates for acid phosphatase, alkaline phosphatase, alanine aminotransferase, amylase, angiotensin converting enzyme, aspartate aminotransferase, creatine kinase, gamma-glutamyltransferase, lipase, lactate dehydrogenase, and glucose-6-phosphate dehydrogenase.
	Z	**Acidic, basic or charged groups**, such as COOH, SO_3H, primary, secondary or tertiary amino groups, nitrogen-heterocycles, ethers, phosphonium groups, quaternary ammonium groups, sulfonium groups, chelated metal ions, tetralky or tetraryl borate or stable carbanions; and **Other Groups**, such as ethers, polyethers, ether diamines, polyether diamines, diamines, amides, polyamides, polythioethers, disulfides, silyl ethers, alkyl or alkenyl chains (straight chain or branched and portions of which may be cyclic), aryl, diaryl or alkyl-aryl groups. Aryl groups in linkers can contain one or more heteroatoms (e.g., N, O or S atoms).
	Q	**Biotin and structurally modified biotins** such as d-iminobiotin; streptavidin or avidin; **1,2-diols**, such as 1,2-dihydroxyethane, 1,2-dihydroxyalkanes cyclic 1,2-dihydroxyalkanes, e.g., 1,2-dihydroxycyclohexane; **Antibody haptens**, such as dinitrophenyl; **metal-binding ligands**, such as (oligomeric histidine); and **glutathione**.
US 2002/0040275	X	**Sulfonyl groups**, such as sulfonates, sulfates, sulfinates, sulfamates, sulfonated alkyl or aryl thiols, sulfonyl halides, sulfonyl esters or any reactive functionality having a sulfur group bonded to two oxygen atoms; **Epoxides**, such as aliphatic, aralkyl, cycloaliphatic and Spiro epoxides, and fumagillin, which is specific for metalloproteases; and **Other groups**, such as alkylating agents, acylating agents, ketones, aldehydes, or phosphorylating agents, fluorophosphonyls, fluorophosphoryls, fluorosulfonyls, alpha-haloketones or aldehydes or their ketals or acetals, respectively, α-haloacyls, nitrites, iodoacetylamides, maleimides, isocyanates, isothiocyanantes, tetrafluorophenyl esters, N-hydroxysuccinimidyl esters, acid halides, acid anhydrides, unsaturated carbonyls, alkynes, hydroxamates, alpha halomethylhydroxamates, aziridines, epoxides, or arsenates and their oxides. **States that "A "chemically reactive group" is a moiety including a reactive functionality that does not react efficiently with the generally available functional groups of proteins, e.g. amino, hydroxy, carboxy, and thiol, but will react with a functionality present in a particular conformation on a surface. In some situations the reactive functionality will serve to distinguish between an active and an inactive protein. In other situations, the conformation of the chemically reactive group will bind to the specific conformation of the target protein(s), whereby with a slowly reactive functionality or one that requires activation, the predominant reaction will be at the active site. For example a photoactivatable group may be used such as a diazoketone, arylazide, psoralen, arylketone, arylmethylhalide, etc. any of which can bind non-selectively to the target protein, while the probe is bound to the active site. Olefins and acetylenes to which are attached electron withdrawing groups such as a sulfone, carbonyl, or nitro group may be used to couple to sulfhydryl groups."**

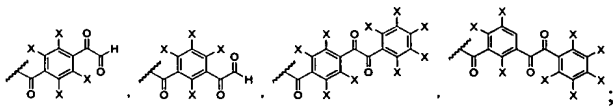
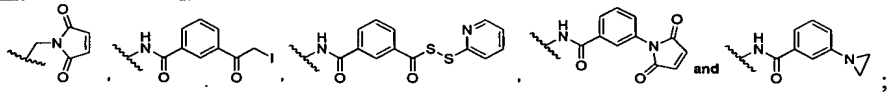
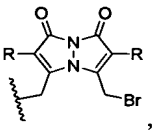
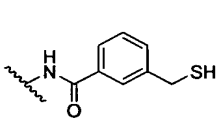
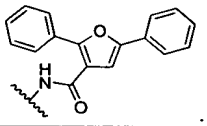
Document	Moiety	Example(s)
US 2002/0192720	Z	**Oligomeric groups**, such as: 1) **polyalkyleneoxy groups**, where alkylene is of from 2-3 carbon atoms; 2) **polymethylene, polyamide, and polyester**, where individual monomers will generally be of from 1 to 6, more usually 1 to 4 carbon 5 atoms; 3) **oligomers of amino acids** (both naturally occurring and synthetic) and oligopeptides that employ amino acids of from 2-3 carbon atoms (i.e. glycine and alanine); 4) **oligonucleotides**, both naturally occurring and synthetic; 5) **condensation polymers** ofmonomeric units; and 6) **polyamides, polythioethers, polyether diamines and polyethers;** **Other groups**, such as 1) **alkylene, alkyleneoxy**; 2) **ethers, diamines, ether diamines, amides, disulfides, silyl ethers, methylene, alkyl or alkenyl chains** (straight chain or branched and portions of which may be cyclic); 3) **aryl, diaryl or alkyl-aryl groups**, having from 0 to 3 sites of aliphatic unsaturation. Aryl groups in linker moieties can contain one or more heteroatoms (e.g., N. O or S atoms); 4) substituted **benzyl ethers, esters, acetals or ketals and diols.**
	Q	**Biotin** and **fluorescein**.
	X	**Peptide Reactive Groups**, such as: 1) **Thiol reactive groups**, such as epoxides, α-haloacyl groups, nitriles, nitriles, sulfonated alkyl or aryl thiols and maleimides; 2) **Amino reactive groups**, such as sulfonyl halides, isocyanates, isothiocyanantes, active esters (including tetrafluorophenyl esters and N-hydroxysuccinimidyl esters), acid halides, acid anyhydrides, aldehydes or ketones in the presence or absence of $NaBH_4$ or $NaCNBH_3$, α-haloacyl groups, iodoacetylamides, and pentafluorophenyl esters; 3) **Carboxylic acid reactive groups**, such as amines or alcohols (in the presence of a coupling agent, such as dicyclohexylcarbodiimide, or 2,3,5,6-tetrafluorophenyl trifluoroacetate and in the presence or absence of a coupling catalyst such as 4-dimethylaminopyridine; and transition metal-diamine complexes, including Cu(II)phenanthroline.) 4) **Other peptide reactive groups**, such as groups that react with sulfydryl, homoserine lactone groups, carboxylate groups, and ester groups; **Ester reactive groups**, such as amines which can react with homoserine lactone; **Phosphate reactive groups**, such as chelated metals, for example Fe(III) or Ga(III), chelated to, for example, nitrilotriacetiac acid or iminodiacetic acid; **Aldehyde or ketone reactive groups**, such as amines treated with $NaBH_4$ or $NaCNBH_3$ that can react with carbohydrates treated with periodate.

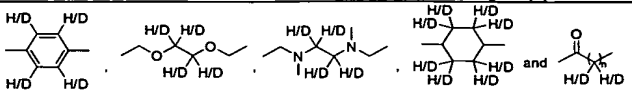
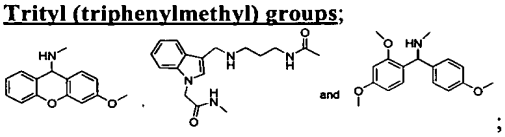
Document	Moiety	Example(s)
	Z	B^1-X^1-$(CH_2)_n$-[X^2-$(CH_2)_m$]$_x$-X^3-$(CH_2)_p$-X^4-B^2-; wherein: X^1, X^2, X^3 and X^4 are independently selected from O, S, NH, NRR'^+, CO, COO, COS, S-S, SO, SO_2, CO-NR', CS-NR', Si-O, aryl groups, diaryl groups or a direct link; B1 and B2 are independently selected from COO, CO, CO-NR', CS-NR', $(CH_2)_q$, $(CH_2)_q$-COO, $(CH_2)_q$-CO, $(CH_2)_q$-CO-NR', $(CH_2)_q$-CS-NR', or a direct link, where n, m, p, q and x are integers from 0 to 100; R is an alkyl, alkenyl, alkynyl, alkoxy or aryl group; R' is a hydrogen, an alkyl, alkenyl, alkynyl, alkoxy or aryl group; One or more of the (CH_2) groups is optionally substituted with C1-C6 alkyl, alkenyl, or alkoxy groups, an aryl group, or functional groups that promote ionization, such as acidic or basic groups or groups carrying permanent positive or negative charge; and one or more single bonds connecting CH_2 groups can be replaced with a double or triple bond; where the moiety optionally contains one or more stable isotopes, such as deuterium and/or optionally contains a cleavable linker; and **Acidic, basic or charged groups**, such as COOH, SO_3H, primary, secondary or tertiary amino groups, nitrogen-heterocycles, ethers, phosphonium groups, quaternary ammonium groups, sulfonium groups, chelated metal ions, tetralky or tetraryl borate or stable carbanions; and **Cleavable Groups**, such as: 1) **photocleavable groups**, such as 1-(2-nitrophenyl) and 1-(2-nitrophenyl)ethyl; 2) **Thermally labile linkers**, such as nucleic acid duplexes (such as, for example nucleic acid/peptide nucleic acid (PNA) duplexes, PNA/PNA duplexes); 3) **Other cleavable linkers**, such as disulfides, acid or base labile groups (such as, for example, diarylmethyl or trimethylarylmethyl groups), silyl ethers, carbamates, oxyesters, thioesters, thionoesters, α-fluorinated amides and esters; and 4) **Enzymatically cleavable linkers**, such as protease sensitive amides or esters, beta-lactamase-sensitive beta-lactam analogs, and linkers that are nuclease-cleavable or glycosidase cleavable. **Other Groups**, such as ethers, polyethers, ether diamines, polyether diamines, diamines, amides, polyamides, polythioethers, disulfides, silyl ethers, alkyl or alkenyl chains (straight chain or branched and portions of which may be cyclic), aryl, diaryl or alkyl-aryl groups. Aryl groups in linkers can contain one or more heteroatoms (e.g., N, O or S atoms).
	Q	**Biotin and structurally modified biotins** such as d-iminobiotin; streptavidin or avidin; **1,2-diols**, such as 1,2-dihydroxyethane, 1,2-dihydroxyalkanes cyclic 1,2-dihydroxyalkanes, e.g., 1,2-dihydroxycyclohexane; **Antibody haptens**, such as dinitrophenyl; **metal-binding ligands**, such as (oligomeric histidine); and **glutathione**.
US 2002/0064799 and WO 01/77668	**X**	**Sulfonyl groups**, such as sulfonates, sulfates, sulfinates, sulfamates, sulfonated alkyl or aryl thiols, sulfonyl halides, sulfonyl esters or any reactive functionality having a sulfur group bonded to two oxygen atoms; **Sulfonate esters**, such as pyridylsulfonates and R-SO_3-, where R is  ; **Epoxides**, such as aliphatic, aralkyl, cycloaliphatic and Spiro epoxides, and fumagillin, which is specific for metalloproteases; and **Other groups**, such as alkylating agents, acylating agents, ketones, aldehydes, or phosphorylating agents, fluorophosphonyls, fluorophosphoryls, fluorosulfonyls, alpha-haloketones or aldehydes or their ketals or acetals, respectively, α-haloacyls, nitrites, iodoacetylamides, maleimides, isocyanates, isothiocyanantes, tetrafluorophenyl esters, N-hydroxysuccinimidyl esters, acid halides, acid anhydrides, unsaturated carbonyls, alkynes, hydroxamates, alpha halomethylhydroxamates, aziridines, epoxides, or arsenates and their oxides.

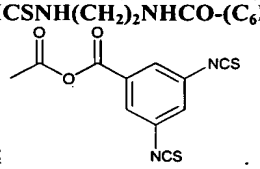
Document	Moiety	Example(s)
	Z	**1) photocleavable groups**, such as 1-(2-nitrophenyl) and 1-(2-nitrophenyl)ethyl; **2) Thermally labile linkers**, such as nucleic acid duplexes (such as nucleic acid/peptide nucleic acid (PNA) duplexes and PNA/PNA duplexes); **3) Other cleavable linkers**, such as disulfides, acid or base labile groups (such as diarylmethyl or trimethylarylmethyl groups), silyl ethers, carbamates, oxyesters, thioesters, thionoesters, α-fluorinated amides and esters; and **4) Enzymatically cleavable linkers**, such as protease sensitive amides or esters, beta-lactamase-sensitive beta-lactam analogs, and linkers that are nuclease-cleavable or glycosidase cleavable. **Other groups**, such as alkylene, alkyleneoxy, ethers, polyethers, diamines, ether diamines, polyether diamines, amides, polyamides, polythioethers, disulfides, silyl ethers, alkyl or alkenyl chains (straight chain or branched and portions of which may be cyclic) aryl, diaryl or alkyl-aryl groups, having from 0 to 3 sites of aliphatic unsaturation; substituted benzyl ethers, esters, acetals or ketals and diols.
	Q	**Biotin and structurally modified biotins** such as d-iminobiotin; **Streptavidin or avidin**; **1,2-diols**, such as 1,2-dihydroxyethane, 1,2-dihydroxyalkanes, cyclic 1,2-dihydroxyalkanes, e.g., 1,2-dihydroxycyclohexane; **Haptens**, such as dinitrophenyl; **Metal-binding ligands**, such as oligomeric histidine; and **Glutathione**.
WO 02/071066; WO 01/77684; and US 2002/0064799	X	**Sulfonyl groups**, such as sulfonates, sulfates, sulfinates, sulfamates, sulfonated alkyl or aryl thiols, sulfonyl halides, sulfonyl esters or any reactive functionality having a sulfur group bonded to two oxygen atoms; **Epoxides**, such as aliphatic, aralkyl, cycloaliphatic and Spiro epoxides, and fumagillin, which is specific for metalloproteases; and **Other groups**, such as alkylating agents, acylating agents, ketones, aldehydes, or phosphorylating agents, fluorophosphonyls, fluorophosphoryls, fluorosulfonyls, alpha-haloketones or aldehydes or their ketals or acetals, respectively, α-haloacyls, nitrites, iodoacetylamides, maleimides, isocyanates, isothiocyanantes, tetrafluorophenyl esters, N-hydroxysuccinimidyl esters, acid halides, acid anhydrides, unsaturated carbonyls, alkynes, hydroxamates, alpha halomethylhydroxamates, aziridines, epoxides, or arsenates and their oxides.
	Z	**alkyleneoxy and polyalkyleneoxy groups**, where alkylene is 1-3 carbon atoms; **amino acids**; **Oligomers and polymers** (generally from about 1 to 10, more usually 1 to 8 monomeric units), such as: **1) polymethylenes, polyamides, polyesters,** and **oligopeptides**, where individual monomers will generally be of from 1 to 6 carbon atoms; **2) oligomers of amino acids** (both naturally occurring and synthetic), **3) oligonucleotides** (both naturally occurring and synthetic); and **4) condensation polymers** of monomeric units. **Other groups**, such as **1) alkylene, alkyleneoxy;** **2) ethers, diamines, ether diamines, amides, disulfides, silyl ethers, methylene, alkyl or alkenyl chains** (straight chain or branched and portions of which may be cyclic); **3) aryl, diaryl or alkyl-aryl groups**, having from 0 to 3 sites of aliphatic unsaturation. Aryl groups in linker moieties can contain one or more heteroatoms (e.g., N. O or S atoms); **4)** substituted **benzyl ethers, esters, acetals or ketals and diols.**

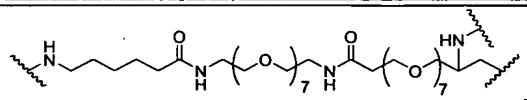
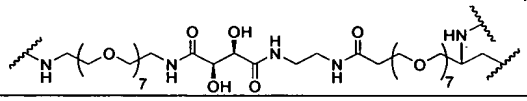
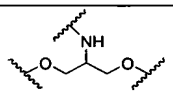
Document	Moiety	Example(s)
	Q	**Antibody haptens**, such dinitrophenyl or receptor haptens; **Metal-binding ligands**, such as oligomeric histidine; glutathione and antibodies; **Biotin, avidin and biotin analogs** such as dethiobiotin and deiminobiotin; **Vicinal diols**, such as 1,2-dihydroxyethane ($HO-CH_2-CH_2-OH$), and other 1,2-dihyroxyalkanes including those of cyclic alkanes, e.g., 1, 2-dihydroxycyclohexane; **Other groups**, such as digoxigenin, maltose, 2,4-dintrobenzene, phenylarsenate, ssDNA, dsDNA, polypeptides, saccharine; **Detectable labels such as:** 1) **electrochemical labels;** 2) **fluorescent moieties,** such as xanthene dyes, naphthylamine dyes, coumarins, cyanine dyes and metal chelate dyes, such as fluorescein, rhodamine, rosamine, BODIPY dyes (FL, TMR, and TR), dansyl, lanthanide cryptates, erbium, terbium and ruthenium chelates, e.g. squarates; energy transfer dyes;. matched dyes (such as those described in U.S. Patent No. 6,127,134); cyanine dyes; BODIPY dyes; conjugated structures comprising a polymethine chain terminating in nitrogen atoms; 3) **conjugated pyran molecules**, including xanthenes; eosin, erythrosin, fluorescein, Oregon green, and various commercially available Alexa Fluor dyes (Molecular Probes, Inc.); and 4) **rhodamine dyes** such as tetramethylrhodamine, 5-carboxytetramethylrhodamine, 6-carboxytetramethylrhodamine, carboxyrhodamine-6G, rhodamine-B sulfonyl chloride, rhodamine-red-X, and carboxy-X-rhodamine.
	Z	**Amino acids**, such as lysine, glutamic acid, cysteine, tyrosine, serine
	Q	**Biotin**
WO 02/063271	**X**	**Sulfonyl groups**, such as sulfonates, sulfates, sulfinates, sulfamates, sulfonated alkyl or aryl thiols, sulfonyl halides, sulfonyl esters or any reactive functionality having a sulfur group bonded to two oxygen atoms; **Epoxides**, such as aliphatic, aralkyl, cycloaliphatic and Spiro epoxides, and fumagillin, which is specific for metalloproteases; and **Other groups**, such as alkylating agents, acylating agents, ketones, aldehydes, or phosphorylating agents, fluorophosphonyls, fluorophosphoryls, fluorosulfonyls, alpha-haloketones or aldehydes or their ketals or acetals, respectively, α-haloacyls, nitrites, iodoacetylamides, maleimides, isocyanates, isothiocyanantes, tetrafluorophenyl esters, N-hydroxysuccinimidyl esters, acid halides, acid anhydrides, unsaturated carbonyls, alkynes, hydroxamates, alpha halomethylhydroxamates, aziridines, epoxides, or arsenates and their oxides;  , wherein R is halogen, such as F, Cl, Br or I;  **fluorophosphonates**; such as , wherein L is $-CH_2-$, -O- or -S-; R is H or an alkyl chain from 1-20 carbon atoms and from 0 to 5 heteroatoms, and may be alkyl alkenyl, or alkynyl (each straight or branched); and may include one or more aromatic, alicyclic, heteroaromatic, or hetrocyclic groups. Preferred –L-R are $-O-CH_3$, $-O-CH_2CH_3$, $-O-CH-(CH_3)_2$, $-CH_3$, $-CH-(CH_3)_2$, and $-CH_2-CH_3$.

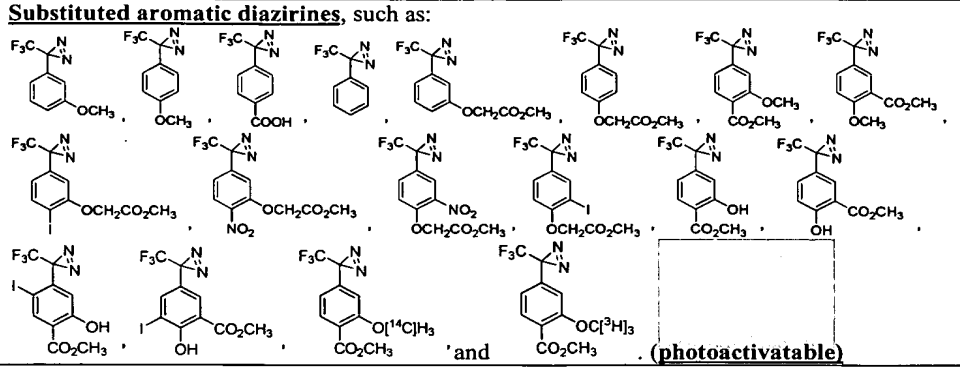
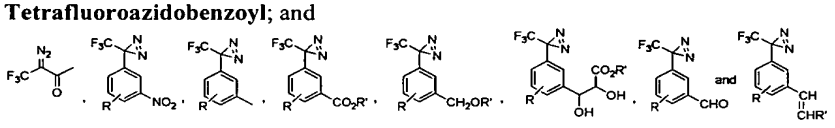
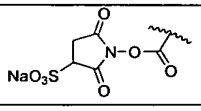
Document	Moiety	Example(s)
	Z	**alkyleneoxy and polyalkyleneoxy groups**, where alkylene is 1-3 carbon atoms; **amino acids**; **Oligomers and polymers** (generally have from about 1 to 10, more usually 1 to 8 monomeric units), such as: **1) polymethylenes, polyamides, polyesters,** and **oligopeptides**, where individual monomers will generally be of from 1 to 6 carbon atoms; **2) polymers of amino acids** (both naturally occurring and synthetic), **3) oligonucleotides** (both naturally occurring and synthetic); and **4) condensation polymers** of monomeric units. **Other groups**, such as **1) alkylene, alkyleneoxy;** **2) ethers, diamines, ether diamines, amides, disulfides, silyl ethers, methylene, alkyl or alkenyl chains** (straight chain or branched and portions of which may be cyclic); **3) aryl, diaryl or alkyl-aryl groups**, having from 0 to 3 sites of aliphatic unsaturation. Aryl groups in linker moieties can contain one or more heteroatoms (e.g., N. O or S atoms); **4)** substituted **benzyl ethers, esters, acetals or ketals and diols.** **5)**  and  ; where n and m are independently an integer from 0 to 8.
	Q	**Fluorescent moieties**, such as: 
WO 02/081752	**X**	**Amino acid reactive groups**, such as: **1) Thiol specific reactive groups**, such as maleimide or pyridyl-dithio, sulfonated alkyl thiols, sulfonated aryl thiols; **2) Amine specific reactive groups**, such as succinimide and malemide; **3) Threonine/Serine specific reactive groups**, such as hydrazide; **Other groups**, such as receptor ligands, antibodies, ATP, GTP, NAD, NADP, NADH, NADPH, ubiquitin, epoxides, α-haloacyl and nitriles.
	Z	**polypeptides**, such as poly-glycine, poly-alanine **Moieties can optionally include:** **1) radiolabels or stable isotopic labels**, such as ^{2}H, ^{13}C, ^{15}N, ^{18}O, and/or ^{34}S; **2) cleavage sites**, such as chemical or enzymatic cleavage sites, vicinal diols, ortho-nitrobenzyl ethers, disulfide bonds, diazo bonds, esters, sulfones, peptides, glycerol esters, phosphoesters, polynucleotides and oligonucleotides; and **3) other groups**, such as O, S, NH, CO, COO, COS, S-S, CH_2, alkyl groups, alkenyl groups, alkynyl groups, alkoxy groups and aryl groups.
	Q	**Biotin** and **magnetic particles**.

Document	Moiety	Example(s)
WO 02/42427	X	**Iodoacetamide**; **N-hydroxysuccinimide**; **1,2-dicarbonyls**, such as  ; wherein X is independently selected from the group consisting of H, D, OH, OD, R, OR, $OSiR_3$, Cl, Br, I, F, SH, SR, NH_2, NHR, and NR_2; R is selected from the group consisting of an optionally substituted: C_1-C_{20} alkyl, C_2-C_{20} alkenyl, C_2-C_{20} alkynyl, including deuterium substitutions; and n=0-10; **Thiol reactive moieties**, such as: 1)  ; and 2) **Bromobimane moieties**, such as  , where R = CH_3, CD_3, C_2H_5, C_2D_5, C_6H_5, or C_6D_5; and **Phosphoprotein reactive moieties**, such as  and  .
	Z	-NH(CH_2CH_2O)$_n$(CH_2)$_2$-NH-
	Q	**Nucleic acids and proteins**, such as protein A, protein G, DNA, enzymes and antibodies; and **Other molecules**, such as biotin, cofactors, lectins, biomimetic dyes, metal ions, phage displays, aptamers, carbohydrates, ATP, benzamide, phenylboronic acid, heparin, receptors, metal binding moieties such as (2,2'-bipyridine) and digoxigenin.
WO 03/005026	X	**Carbohydrate-reactive moieties**, such as amines and hydrazines; **Other moieties**, such as fluorophosphonates, α-halo or (acyloxy)methyl ketones, 4-(phenylamino)quinazolines, 4-(phenylamino)pyrido moieties, enzyme substrates, maleimide, pyridyldisulfide, vinylsulfone, iodoacetamide, epoxide, nitrile, aryl thiol, sulfonated alkyl, N-hydroxysuccinimide ester, isothiocyanates, isocyanates, imidoesters or sulphonyl halides, aldehydes, ketones, alcohols and thiols.

Document	Moiety	Example(s)
	Z	 where H/D indicates that hydrogen can be optionally replaced with deuterium; **Detectable moieties**, such as: **1) chromophores,** **2) azo dyes,** **3) fluorophores,** such as, fluoresceins, rhodamines, coumarins, cyanine dyes, BODIPY dyes and squarate dyes and luminescent molcules; **Trityl (triphenylmethyl) groups;**  **Other groups**, such as ethers, polyethers, ether diamines, polyether diamines, diamines, amides, polyamides, polythioethers, disulfides, silyl ethers, alkyl or alkenyl chains (straight chain (such as C4 alkyl or C6 alkyl) or branched and portions of which may be cyclic), aryl, diaryl or alkyl-aryl groups. Aryl groups in linkers can contain one or more heteroatoms (e.g., N, O or S atoms); and **Moieties can include**: **1) cleavable groups**, such as acid cleavable groups, base labile groups, groups cleavable with fluoride ions, nitrobenzophenones, 3-(aminomethyl)-indol-1-yl-acetic acid, 9(aminoxanthen-3-yl)oxy acetic acid and 4-[amino-(2,4-dimethoxyphenyl)-methyl]-phenol. **2) stable isotopes** such as, for example ^{2}H, ^{13}C, ^{15}N, ^{17}O, ^{18}O, ^{34}S, ^{33}S, ^{36}S, **3) radioisotopes** such as, for example ^{3}H, ^{14}C and ^{35}S; **4) modifications to enhance ionization**, such as: **a) acidic or basic groups**, such as COOH, SO_3H, primary, secondary or tertiary amino groups, nitrogen-heterocycles, ethers, or combinations of these groups; and **b) charged groups**, such as phosphonium groups, quaternary ammonium groups, sulfonium groups, chelated metal ions, tetralky or tetraryl borate or stable carbanions.
	Q	**Biotin**
Alley *et al.*, "Mapping protein-protein interactions in the bacteriophage T4 DNA polymerase holoenzyme using a novel trifunctional photo-cross-linking and affinity reagent" *J. Am. Chem. Soc.* 122:6126-6127 (2000)	**X**	**2-thiopyridine; aryl azides (photoactivatable); and N-Hydroxysulfosuccinimide (Sulfo-NHS) esters**
	Z	**Lysine**
	Q	**Biotin**
Bogyo *et al.*, "Covalent modification of the active site threonine of proteasomal beta subunits and the Escherichia coli homolog HslV by a new class of inhibitors," Proc. Natl. Acad. Sci. USA 94(13):6629-6634 (1997); and Bogyo *et al.*, "Substrate binding and sequence preference of the proteasome revealed by active-site-directed	**X**	**Vinyl sulfones and phenolic vinyl sulfones**
	Z	**n/a**

Document	Moiety	Example(s)
affinity probes," Chem. Biol.5(6):307-320 (1998).	**Q**	**Biotin** and **4-hydroxyl-5-iodo(^{125}I)-3-nitrophenyl acetate**
Boring *et al.*, "Trifunctional agents as a design strategy for tailoring ligand properties: irreversible inhibitors of A1 adenosine receptors", *Bioconjug Chem.*, 2(2):77-88 (Mar-Apr 1991)	**X**	**Phenylisothiocyanates; N-hydroxysuccinimide esters; $CONH(CH_2)_2NHCOCH_2Br$; $NHCSNH(CH_2)_2NHCO$-(C_6H_3-2-NO_2-5-N_3)-; $NHCSNH(CH_2)_2NHCO$-(C_6H_3-2-OH-4-N_3)-;**  and
	Z	**Benzene** and **pyridine**
	Q	**Biotin**; **Spin labels**, such as TEMPO; and **Fluorescent labels**, such as fluorescein isothiocyanate (FITC) or 4-nitrobenz-2-oxa-1,3-diazole (NBD).
Dormán *et al.*, "Chemical library approaches to target validation in the post-genomic era," Curr. Drug Discov. October:21-24 (2001).	**X**	**Photoactivatable moieties:** 1) **Benzophenone;** 2) **Phenyl azide; and** 3) **Trifluoromethylphenyl diazirine.**
	Z	**n/a**
	Q	**n/a**
Dormán *et al.*, "Using photolabile ligands in drug discovery and development," Trends Biotechnol. 18:64-77 (2000).	**X**	**Maleimide**; **Photoactivatable moieties**: 1) **Benzophenones;** 2) **Tetrafluorophenyl azides**; and 3) **Trifluoromethylphenyl diazirines.**
	Z	**n/a**
	Q	**n/a**
Fang *et al.*, "A bifunctional photoaffinity probe for ligand/receptor interaction studies," J. Am. Chem. Soc. 120: 8543-8544 (1998)	**X**	**3-(trifluoromethyl)-3-phenyldiazirine (photoactivatable)**
	Z	**lysine; philanthotoxin-433**
	Q	**Biotin**
Gygi *et al.* Quantitative analysis of complex protein mixture using isotope-coded affinity tags" Nature Biotech. 17, 994-999, (1999)	**X**	**α-iodo acetamide**
	Z	**$-(CH_2)_2-(CH_2-O-CH_2)_3-(CH_2)_2-$**
	Q	**Biotin**
Hagenstein *et al.*, "Affinity-based tagging of protein families with reversible inhibitors: a concept for functional proteomics," Angew. Chem. Int. Ed. 42: 5635-5638 (2003).	**X**	**4-benzoylphenylalanine (photoactivatable)**
	Z	**Triethylenetetramine**
	Q	**Fluorescein**

Document	Moiety	Example(s)
Hasegawa *et al.*, "Determination of the binding site on the extracellular domain of guanyl cyclase C to heat-stable enterotoxin," J. Biol. Chem. 274(44):31713-31719 (1999).	**X**	**Phenyl azide (photoactivatable)**
	Z	**n/a**
	Q	**n/a**
Hashimoto *et al.*, "Cell-surface recognition of biotinylated Membrane Proteins Requires Very long Spacer Arms: An Example from Glucose-Transporter Probes" *Chembiochem* 52-59 (2001)	**X**	**Trifluoromethylphenyl diazirine (photoactivatable)**
	Z	 , and 
	Q	**Biotin**
Hashimoto *et al.*, "Synthesis of biotinylated bis(D-glucose) derivatives for glucose transporter photoaffinity arbine " *Carbohydrate Research* 331:119-127 (2001)	**X**	**Trifluoromethylphenyl diazirine (photoactivatable)**
	Z	**$-CH_2-(CH_2OCH_2)_2-CH_2-$** and 
	Q	**Biotin**
Hatanaka *et al.*, "A Carbine-generating biotinylated lactosylceramide analog as novel photoreactive substrate for GM3 synthase" *Bioorg. Med. Chem. Lett.* 5(23):2859-2862 (1995)	**X**	**Trifluoromethylphenyl diazirine (photoactivatable)**
	Z	**Lysine**
	Q	**Biotin**
Hatanaka *et al.*, "A Novel Biotinylated Heterobifunctional Cross-linking Reagent Bearing an Aromatic Diazirine" *Bioorg. Med. Chem. 2(12):1367-1373* (1994)	**X**	**Trifluoromethylphenyl diazirine (photoactivatable)** and **N-hydroxysuccinimide**
	Z	**$-CH_2-(CH_2OCH_2)_2-CH_2-NH-$,**
	Q	**Biotin**

Document	Moiety	Example(s)
Hatanaka *et al.*, "A Novel Family of Aromatic Diazirines for Photoaffinity Labeling" *J. Org. Chem.* 59:383-387 (1994)	X	**Substituted aromatic diazirines**, such as:  and . **(photoactivatable)**
	Z	**n/a**
	Q	**n/a**
Hatanaka *et al.*, "A rapid and efficient method for identifying photoaffinity biotinylated sites within proteins," J. Am. Chem. Soc. 120: 453-454 (1998).	X	**3-(trifluoromethyl)-3-phenyldiazirine (photoactivatable)**
	Z	**Asparagine-NH_2;** **-$(CH_2)_2$-O-CH_2-CH_2-O-$(CH_2)_2$-**
	Q	**Biotin**
Hatanaka *et al.*, "Photoaffinity labeling in drug discovery and developments: chemical gateway for entering proteomic frontier," Curr. Top. Med. Chem. 2: 271-288 (2002).	X	**Photoactivatable moieties**: **1) Phenylazide**; m-nitro-phenylazide; m-hydroxy-phenylazide **2) Carbonyl**; **3) Diazirines**, such as 3-aryl-3-trifluoromethyldiazirines; **4) Diazo**; **5) Perfluorophenylazides**; **6) 4-benzoylphenylalanine**; **7) Benzophenone**; **8) Tetrafluoroazidobenzoyl**; and **9)**  ;wherein R and R' are independently selected from –H and –OCH_3.
	Z	**-$(OCH_2CH_2)_3NH$-;** **ethylene**; and **-NH-CH(CH_2-O-)$_2$.**
	Q	**Biotin**
Horney *et al.*, "Synthesis and characterization of Insulin-like Growth Factor (IGF)-1 photoprobes selective for the IGF-binding proteins (IGFBPs)" *J. Biol. Chem.* 276(4):2880-2889 (2001)	X	**Arylazides (photoactivatable)** and 
	Z	**Lysine**
	Q	**Biotin**
Ilver *et al.*, "Helicobacter pylori Adhesin Binding Fucosylated Histo-Blood Group Antigens Revealed by Retagging," *Science* 279:373-377 (1998).	X	***N*-hydroxysuccinimide esters**, and **aryl azides (photoactivatable)**
	Z	**n/a**
	Q	**Biotin**
Jeffery *et al.*, "Chemical proteomics and its	X	**Epoxides; fluorophosphonates; sulfonate esters; α-iodo acetamidyl; difluoromethyl phenylphosphates; sulbactams; and aryl sulfonyl fluorides.**

Document	Moiety	Example(s)
application to drug discovery," Curr. Opin. Biotechnol. 14: 87-95 (2003).	Z	**Alkyl; polyethylene glycols; and peptides.**
	Q	**Biotin;** **Detectable moieties, such as:** **1) Fluorescent labels**, such as BODIPY and Alexa Fluors; and **2) Radioactive labels.**
Kam *et al.*, "Biotinylated isocoumarins, new inhibitors and reagents for detection, localization and isolation of serine proteases," Bioconjugate Chem. 4(6):560-567 (1993).	X	**α-chloro ketones; diazomethyl ketones; isocoumarins and isocoumarin derivatives.**
	Z	**-NH-$(CH_2)_2$NHCOCH$_2$NHCO-; 6-aminocaproyl (Aca); and $(Aca)_2$.**
	Q	**Biotin**
Konoki *et al.*, "Development of Biotin-Avidin Technology to Investigate Okadaic Acid-Promoted Cell Signaling Pathway" *Tetrahedron* 56:9003-9014 (2000)	X	**Aryl(trifluoromethyldiazirines (photoactivatable)**
	Z	**-$CH_2(CH_2OCH_2)_2CH_2$-** and **-$NH(CH_2)_5NH$-**
	Q	**Biotin**
Koumanov *et al.*, "Cell-surface biotinylation of GLUT4 using bis-mannose photolabels" *Biochem J.* 330:1209-1215 (1998)	X	**Aryl(trifluoromethyldiazirines (photoactivatable)**
	Z	**-$CH_2(CH_2OCH_2)_2CH_2$-; -$NH(CH_2)_5NH$-**
	Q	**Biotin**
Laktionov *et al.*, "Characterization of membrane oligonucleotide-binding proteins and oligonucleotide uptake in keratinocytes," Nucl. Acids. Res. 27(11):2315-2324 (1999).	X	**4-[(N-2-chloroethyl-N-methyl)amino]benzylamine** and ***N*-hydroxysuccinimide esters**
	Z	**n/a**
	Q	**Rhodamine**
Laktionov *et al.*, "Interaction of oligonucleotides with cellular proteins," Nucleos. Nucleot. Nucl. 20(4-7):859-862 (2001).	X	**4-[(N-2-chloroethyl-N-methyl)amino]benzylamine**
	Z	**n/a**
	Q	**n/a**
Lin *et al.*, "Design and synthesis of a novel photoaffinity taxoid as a potential probe for the study of paclitaxel-microtubules interactions," Tetrahedron Letters 41:4287-4290 (2000)	X	**3-nitro-5-trifluoromethyldiazirinyl-phenoxyacetyl (photoactivatable)**
	Z	**-$CH_2(CH_2OCH_2)_2CH_2$-**
	Q	**Biotin**
Liu et al., "Activity-based protein profiling:	X	**Fluorophosphonates**

Document	Moiety	Example(s)
the serine hydrolases," Proc. Natl. Acad. Sci. USA 96(26):14694-14699 (1999).	**Z**	**Alkyl chains**
	Q	**Fluorescein and biotin**
Nazif *et al.*, "Global analysis of proteasomal substrate specificity using positional-scanning libraries of covalent inhibitors," Proc. Natl. Acad. Sci. USA 98(6):2967-2972 (2001).	**X**	**Vinyl sulfones**
	Z	**n/a**
	Q	**n/a**
Nesnas *et al.*, "Synthesis of biotinylated retinoids for cross-linking and isolation of retinol binding proteins," Tetrahedron 58:6577-6584 (2002)	**X**	**Chloroacetates, bromoacetates**
	Z	**Boc-lysine**
	Q	**Biotin**
Rühl *et al.*, "A trifunctional reagent for photoaffinity labeling," Tet. Lett. 41: 4555-4558 (2000).	**X**	**Photoactivatable moieties**: **1)** 3-(3-nitrophenyl)-3-(trifluoromethyl)-diazirine; and **2)** 3-(3-methoxyphenyl)-3-(trifluoromethyl)-diazirine.
	Z	**Isoserine**
	Q	**Biotin**
Rühmann *et al.*, "Synthesis and characterization of a photoactivatable analog of corticotrophin-releasing factor for specific receptor labeling," Proc. Natl. Acad. Sci. USA 93:10609-10613 (1996)	**X**	**3-(trifluoromethyl)-3-phenyldiazirine (photoactivatable)**
	Z	**Tyrosine**
	Q	**n/a**
Santhoshkumar *et al.*, "Identification of a region in alcohol dehydrogenase that binds to alpha-crystallin during chaperone action" *Biochimica et Biophysica Acta* 1598:115-121 (2002)	**X**	**Sulfo-N-hydroxysuccinimidyl esters, phenyl azides (photoactivatable)**
	Z	**Lysine**
	Q	**Biotin**
Sugimoto *et al.*, "Syntheses of novel photoaffinity probes for	**X**	**Photoactivatable moieties**: **1)** Trifluromethylphenyl diazirines, and **2)** phenyl azides

Document	Moiety	Example(s)
bioorganic studies on nyctinasty of leguminous plants" *Tet. Lett.* 43:6529-6532 (2002)	**Z**	**-$(CH_2CH_2O)_3$-, alkyl moieties**
	Q	**Biotin**
Yang *et al.*, "Development of high-affinity ligands and photoaffinity labels for the D-fructose transporter GLUT5" *Biochem. J.* 367:533-539 (2002)	**X**	**Photoactivatable moieties:** **1) Trifluromethylphenyl diazirines, and** **2) phenyl azides**
	Z	**-$NH(CH_2)_5CONH(CH_2CH_2O)_3$-, and** **-$(NH(CH_2)_2NHCO(CH(OH))_2CONH(CH_2CH_2O)_3$-**
	Q	**Biotin**
Zhou *et al.*, "Quantitative proteome analysis by solid-phase isotope tagging and mass spectrometry," Nat. Biotechnol. 19: 512-515 (2002).	**X**	**Iodoacetyl**
	Z	**n/a**
	Q	**n/a**

The Examiner states regarding the state of the prior art and also predictability:

> . . . the predictability in the art is low when the full scope of the claims is taken into consideration. For example, Lauf *et al.* state, "The preparation of new materials with novel and useful chemical and/or physical properties is at best unpredictable considering current levels of understanding. Consequently, the discovery of new materials depends largely on the ability to synthesize and analyze new compounds. Given approximately 100 elements in the periodic table, which can be used to make compositions consisting of three, four, five, six or more elements, the universe of possible new compounds remains largely unexplored" (e.g., see U.S. Patent Application Pub. No. 2004/0062911 Al, page 1, paragraph 4). Thus, **the presently claimed compounds** by analogy "remain largely unexplored" because they could be constructed of any conceivable combination of elements in the periodic table. Furthermore, although organic chemistry (i.e., compounds restricted to a limited number of elements in the periodic table) is a mature art, it is not sufficiently developed to permit the synthesis of any pharmaceutical drug, drug fragment, drug intermediate, drug metabolite, etc. For example, Keaslin *et al.* state, "many natural products [which would include pharmaceutical drugs, drug fragments, drug intermediates, drug metabolites, etc.] have complex structures, and, as a result, are currently ... impossible to synthesize" (e.g., see Keasling *et al.*, US Patent Application No. 2006079476, paragraph 6).

The above discussion shows that one of skill in the art can readily identify suitable X, Z and Q moieties to prepare capture compounds that meet the limitations in the claims. Each of X, Z and Q have particular properties that the claims recite; the specification teaches these properties and examples of each, and the skilled artisan is very familiar with each moiety and suitable selections. Regarding drugs, the claims do not require the artisan to synthesize any

drug; the drug is a user selected. The skilled artisan will have the drug of interest available. Again, the claims are directed to methods of identifying non-targets of drugs, not to compounds. Again, it would be absurd to limit methods of assessing drugs to the half dozen or so discussed in the application in order to exemplify the method. As discussed above, the skilled artisan is very familiar with small molecule drugs. Again the claims are directed to methods, not to compounds. To practice the methods, the skilled artisan has to practice the steps of the methods and attach their drug of interest to a capture compound with a trifunctional core that also presents a sorting/immobilization group and a group that upon activation covalently binds to proteins. There is no need to resort to the universe of unexplored compounds.

The Examiner also states:

> Furthermore, it is unclear how a sufficient time for reaching equilibrium between the biomolecule and the capture compound can be achieved without the use of a photoactivatable X group or, alternatively, an X group that can be activated by a change in (see above). Reaching equilibrium takes time and an X group that is constitutively activated would not permit such a waiting period. A constitutively activated X group would react immediately with the target before equilibrium could be achieved between the drug and the biomolecule. Thus, Applicants were not in possession of any X group but, rather, only a select number of "activatable" groups (e.g., see Example 15 wherein a photoactivatable group was used to take a "snap shot" of the reaction at the time of photolysis).

The claim clearly recites that X is photoactivatable (although any activatable X group that covalently binds to proteins could be used). Those of skill in the art can employ known activatable groups in the method. As established above, those of skill in the art are familiar with such groups.

d. Teachings in the application and the presence of working examples

As discussed above, the methods claimed in this application are methods for assessing interactions of selected molecules or moieties (Y) with biomolecules in a particular cell, tissue or other sample. The instantly claimed embodiments are those in which Y is a drug, drug fragment, drug metabolite, prodrug. The method involves providing a compound(s) that present molecules or moieties Y to identify biomolecules in a sample with which Y interacts The X moieties are selected to covalently bind upon activation of the X to capture biomolecules that interact with Y. For example, if Y is a drug, then contacting it with sample from a biological fluid or cell sample can assess with what else, in addition to the drug target, the drug interacts. The non-targets can be responsible, for example, for side-effects.

Dependent claims include further steps of redesigning the drug to eliminate or reduce interactions with non-targets, and thereby reduce undesirable side-effects.

In practicing the methods, the capture compound or compounds are contacted with a sample, such as a cell or tissue sample, under conditions such that the interactions with Y reach equilibrium. The compounds are then exposed to conditions in which X covalently binds or binds with high affinity to the biomolecules that interact with Y and capture them. The captured biomolecules can then be identified. For example, if Y is a drug, a drug typically has a target. When contacting the compound(s) with a cell or tissue sample, Y should interact with its target. In addition, in this method, the capture compounds also will capture non-targets with which Y interacts. Identification of such non-targets can be used to predict side-effects and to aid in design of drugs that have reduced interaction with non-targets, and, hence fewer side-effects.

The compounds also include a Q moiety for sorting compounds, such as by capturing them on a solid support, and optionally include a W moiety that can alter solubility properties of compounds so that they can interact with biomolecules in a hydrophobic environment, such as a cell membrane, or a hydrophilic environment. Hence the instant methods can probe selected environments depending upon properties of the capture compounds conferred by W.

Also, as noted, the method is designed to assess biomolecules, such as proteins, that interact with Y groups, such as drugs, drug fragments, drug metabolites. The Y group interacts with molecules in a sample and reaches equilibrium. X is selected to form a covalent bond, such as by activation with the biomolecule with which Y interacts. This permits assessment of the molecules with which Y interacts. This can be used, for example, to identify so-called non-drug targets and to assess affinities of a drug with its target and non-targets. A particular drug is designed to target a molecule, such as a receptor or ligand. Interaction of a drug or its metabolites or fragments interact with other molecules besides the target can lead to side effects. The method permits identification of biomolecules with which such drugs, metabolites or fragments interact, thereby permitting redesign of drugs to reduce or alter such interactions and/or study of drugs to identify targets.

For practicing the method, Q, X and Y, as well as Z, can be selected as described in the application. Selection of Y depends upon the molecule(s) whose interactions is/are selected for assessment; thusY is user selected. For practicing the method, X and Q, as well as Z, can be selected as described in the application (see pages 54-112, which describe X, Q and Z moieties). The application provides a detailed description of exemplary Z molecules

(pages 54-74), X moieties (pages 74-87), including a description of activatable moieties, and Q moieties (page 108-112), and provides examples of the practice of the method at for example, at page 92- page 93, page 94, line 29, - page 102, and in Example 16.

The pending claims are based on the detailed description in the application, the original claims, description in the Examples, including Examples 14 and 16, and the depiction in Figure 30 and data in Figures 31-38. The original claims set forth the method. Example 30 depicts the method. Example 14 and Figures 31-38 describe and show practice of steps of the method. Disclosure in the specification describes the method and provides exemplary capture compounds that present a variety of drugs. Example 16 explains in great detail how particular family of drugs can be studied using the method. Example 16 shows the structure of capture compounds used in the method and also identifies metabolites and capture compounds containing metabolites. The Example identifies cells from which extracts and be prepared and incubated with the capture compounds, as in Figures 33-38. The captured products can be detected as in Figures 33-38. Therefore, the application provides detailed description for practice of the methods as claimed, including detailed Examples. The application teaches and demonstrates how to practice the method as claimed and shows that capture compounds prepared as described can be used to capture molecules that interact with a Y moiety that is user selected, such as a drug or drug metabolite or drug fragment.

The specification details embodiments in which Y is molecule, such as a drug fragment, intermediate, metabolite or prodrug thereof whose non-targets in a sample are to be identified. For example at pages 6-7 of the specification, which describe embodiments in which the drugs molecules are presented on the capture compounds to determine molecules in a sample that interact therewith to identify non-targets as well as targets:

> The capture compounds, collections and methods provided herein also permit screening of biomolecules, including but not limited to receptor proteins and enzymes, which are drug targets and non-targets, as defined herein, that interact with pharmaceutical drugs under physiological conditions. The screening of biomolecules provides increased understanding of the mechanism of action of the pharmaceutical drugs or drug fragments, metabolites or synthetic intermediates in the drug syntheses, thereby helping the design of more target specific drugs. The methods also provide for identification of non-target biomolecules, such as proteins including but not limited to receptors and enzymes, that interact with pharmaceutical drugs, thereby causing side effects and other undesired therapeutic effects. In one embodiment, various attachments of the drugs or drug fragments, metabolites or synthetic intermediates in the drug syntheses to the capture compounds are used to determine which functionalities of the drugs or drug fragments, metabolites or synthetic intermediates in the drug syntheses interact with the target and non-target biomolecules. In one embodiment, the non-target functionalities are then eliminated from the drug, resulting in an improved drug that exhibits fewer side effects. In another embodiment, a drug is included in the capture compound, proteins that interact with the drug are isolated and identified, the proteins are related to function, and the drug

is re-engineered to eliminate or reduce interactions with non-target proteins. The method may be repeated on the re-engineered drug, as desired.

The specification at page 100, *et seq.*, states:

In certain embodiments the selectivity function [Y] is selected from pharmaceutical drugs or drug fragments set forth below, where attachment of exemplary pharmaceutical drugs to a central core is shown below. In other embodiments, the selectivity function is a drug, drug fragment, drug metabolite, or a drug synthetic intermediate.

The pharmaceutical drugs or drug fragments can be attached to the central core Z, in different orientations via different points of attachment, thereby modulating the selectivity of the capture compound. The attachment of a drug/drug fragment to the central core can be carried out by methods known to a person with skill in the art. Attachment of some exemplary pharmaceutical drugs at various points, to the central core Z is set forth below.

In another embodiment, the capture compounds provided herein include those where the selectivity function is a drug, drug fragment, drug metabolite or a prodrug. In these embodiments, the capture compounds also contain a reactivity function, as defined elsewhere herein. In further embodiments, the capture compounds also contain a sorting function, as defined elsewhere herein.

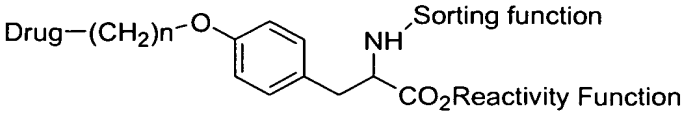
In certain embodiments, the capture compounds that contain drug, drug fragment, drug metabolite or prodrug selectivity functions contain an amino acid core. In one embodiment, the amino acid core may be an amino acid that does not have a functionality on the side chain for attachment of a third function. Such amino acid cores include, but are not limited to, glycine, alanine, phenylalanine and leucine. In these embodiments, the capture compound contains a reactivity function and a selectivity function, which are attached to the amino and carboxy groups of the amino acid.

On the following pages (pages 101-108), the specification provides examples of capture compounds in which Y is drug and exemplifies linkage of exemplary small molecule drugs to a capture compound:

In another embodiment, the amino acid core may be an amino acid that possesses a functionality on the side chain for attachment of a third function. Such amino acid cores include, but are not limited to, serine, threonine, lysine, tyrosine and cysteine. In these embodiments, the capture compound contains a reactivity function, a sorting function and a selectivity function, which are attached to the amino, carboxy and side chain functional groups of the amino acid.

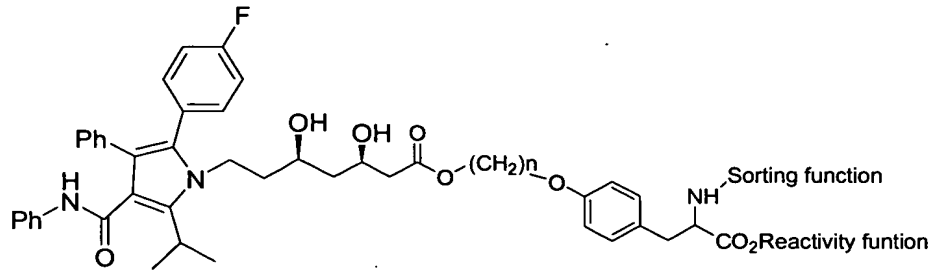
In another embodiment, the amino acid core may be an amino acid that possesses a functionality on the side chain for attachment of a third function. Such amino acid cores include, but are not limited to, serine, threonine, lysine, tyrosine and cysteine. In these embodiments, the capture compound contains a reactivity function, a sorting function and a selectivity function, which are attached to the amino, carboxy and side chain functional groups of the amino acid.

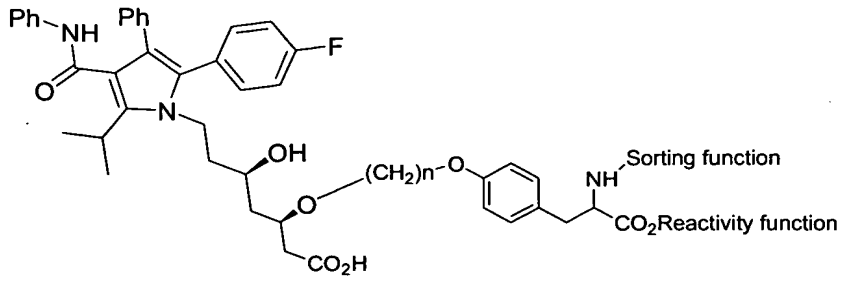
In one embodiment, the core is tyrosine and the capture compounds have the formula:

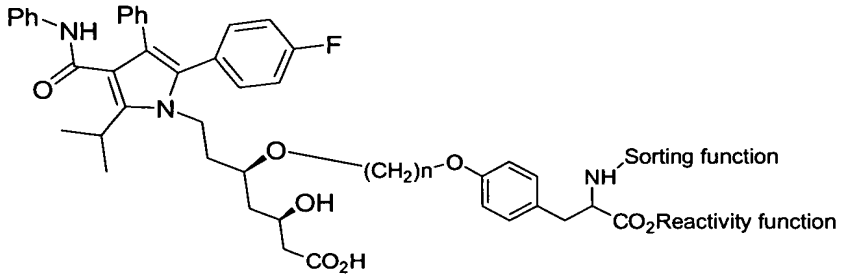


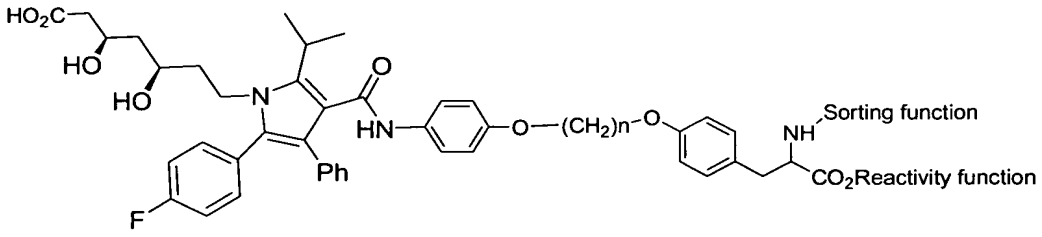
where "drug" refers to a drug, drug fragment, drug metabolite or prodrug.

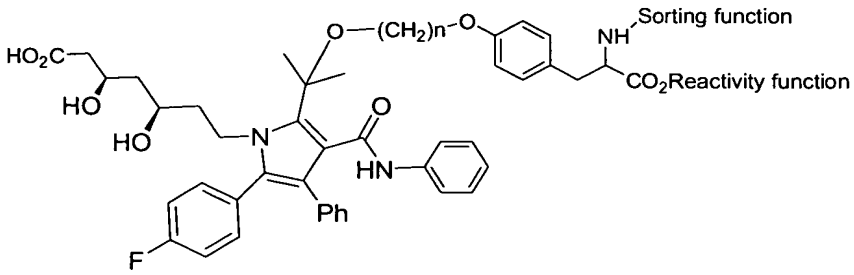
In one embodiment, the drug is LIPITOR® (atorvastatin calcium) and the capture compounds have the formulae:



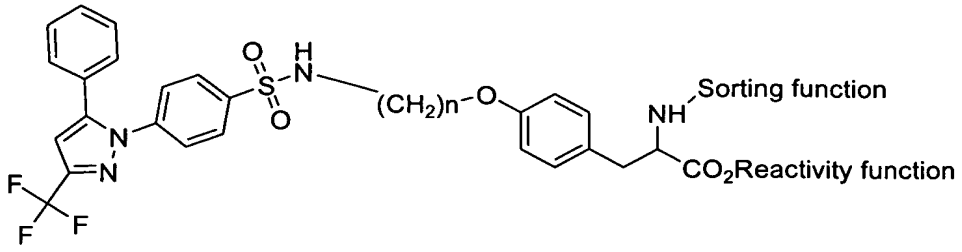


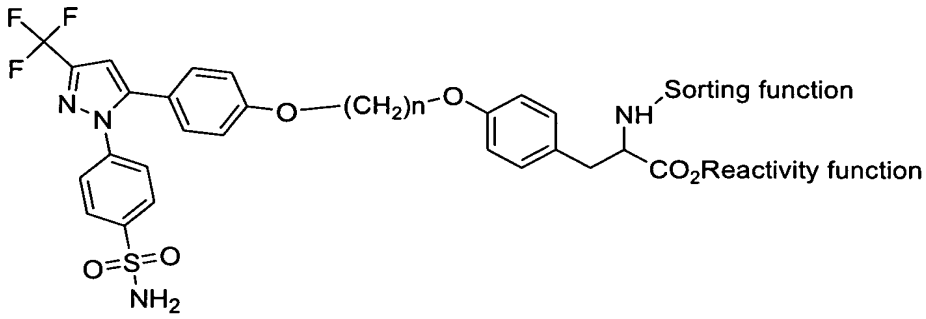


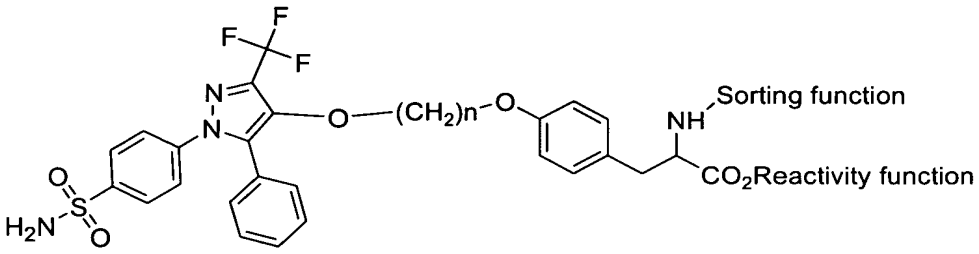




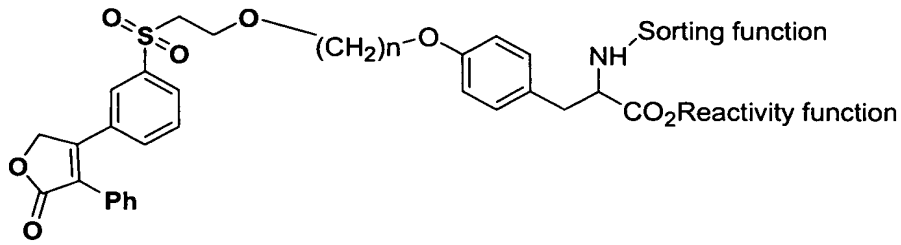
In other embodiments, the drug is CELEBREX® (celecoxib) and the capture compounds have the formulae:

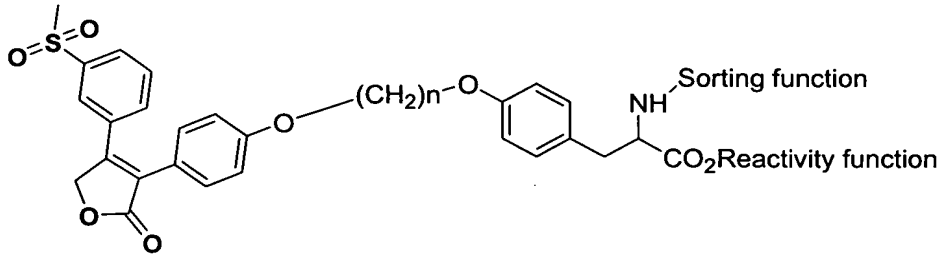


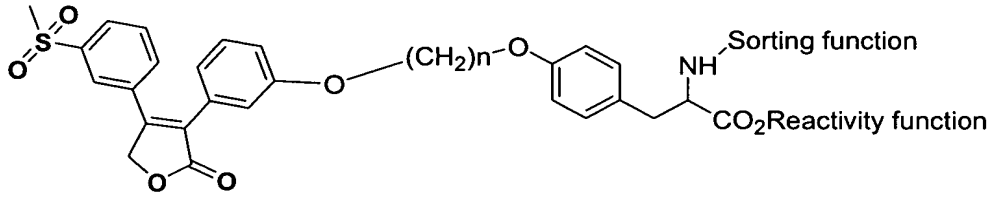


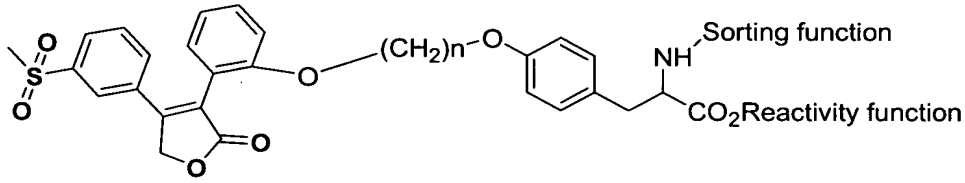


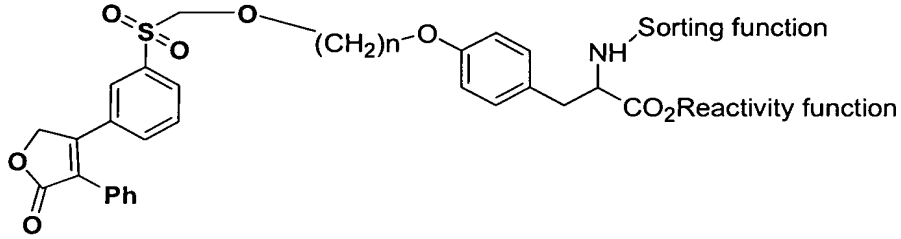
In another embodiment, the drug is VIOXX® (rofecoxib) and the capture compounds have the formulae:



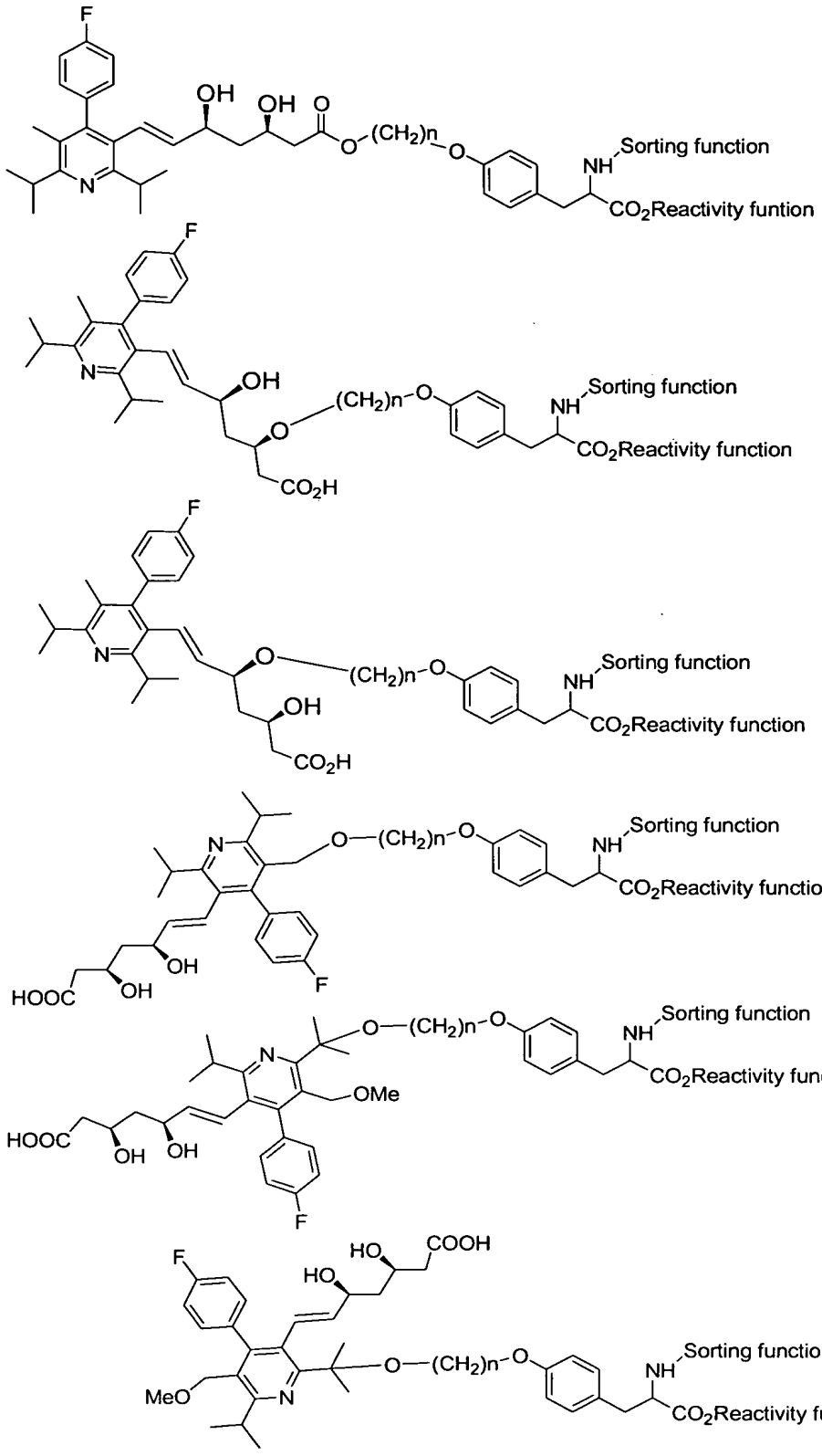








In another embodiment, the drug is BAYCOL® (cerivastatin sodium) and the capture compounds have the formula:


F
OH
OH
O
O
(CH2)n
O
NH
Sorting function
CO2Reactivity funtion
F
N
OH
(CH2)n-O
O
NH
Sorting function
CO2Reactivity function
CO2H
F
N
O
(CH2)n-O
NH
Sorting function
CO2Reactivity function
OH
CO2H
N
O—(CH2)n-O
NH
Sorting function
CO2Reactivity function
OH
HOOC
OH
F
O—(CH2)n-O
NH
Sorting function
N
CO2Reactivity function
OMe
HOOC
OH
OH
F
HO
COOH
F
HO
O—(CH2)n-O
NH
Sorting function
MeO
N
CO2Reactivity function

In another embodiment, the drug is methotrexate and the capture compounds have the formulae:

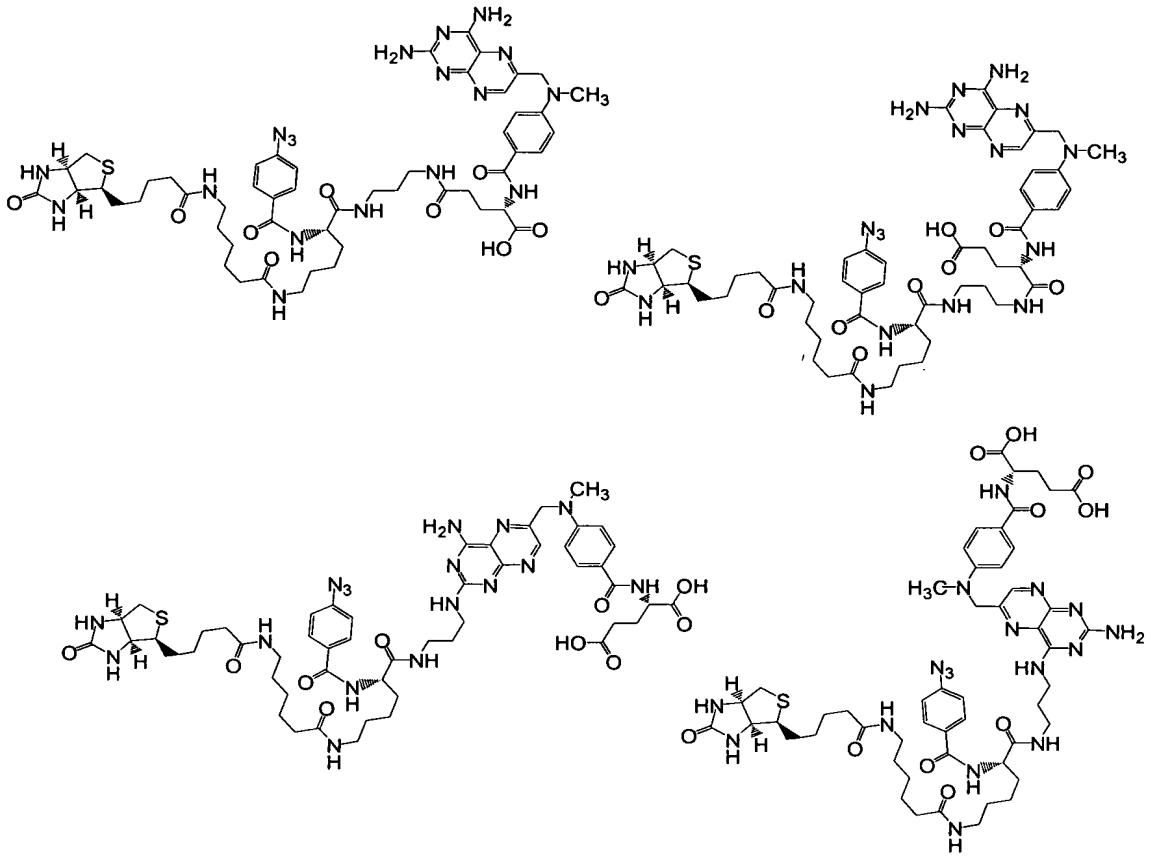


Figure 30 and pages 49-50 and in Example 16 describe the method as claimed:

> In another embodiment, the analytical process (Figure 30) is simple and highly amenable to automation. First, a protein mixture from the cells of interest is incubated with a capture compound in buffer conditions which retain the native structural features of the proteins. The selectivity function [Y] reversibly interacts and comes to equilibrium with those proteins for which it has an affinity. The reactivity function [X] then forms a covalent bond irreversibly linking the compound to those proteins for which there was an affinity

The working Examples teach how to practice these methods and provide data showing practice each step of the method, namely: (1) contacting a capture compound with user-selected Y groups with a sample for a sufficient time for the interaction between the capture compounds and the biomolecules to reach equilibrium, (2) activating X to form a covalent linkage or high affinity bond with a biomolecule to effect capture thereof; and (3) identifying captured molecules is described and exemplified in the application.

Preparation of compounds and reactions in which molecules are captured and analyzed are detailed in the Examples. Examples 12 and 13 show how to make exemplary

capture compounds for use in the method, and Example 14 shows that how to use a capture compound that presents a drug to capture a target molecule in a sample. Example 14 shows that an exemplary capture compound that presents a drug captures target (CAII) and non-target molecules in a sample. Example 15 provides another use for the methods. Example 16 exemplifies all steps in the method and describes use of the method for comparison of drugs in a particular class. Figure 30 and Example 16 describe in detail how to apply such to the instantly claimed method steps. The results in Figures 31-38 demonstrate reduction to practice of the method as claimed. Thus, all steps in the method are described and/or exemplified in the application.

The specification provides a detailed description of how to perform the method as claimed; the claims are not directed to new drugs but to a method whose steps are taught in great detail in the specification. Each step is well-within the level of skill in the art. The application teaches and has working examples detailing how to prepare capture compounds and incubate them and how to identify captured molecules, as claimed. The specification includes Figure 30, which schematically depicts the method, and Example 16 which is a step-by-step example for identifying structural features that contribute to pharmacologic/therapeutic profile and differences in activity within an exemplary class of drugs, the thiazolidinediones (such as Troglitazone (Rezulin™), Rosiglitazone (Avandia™) and Pioglitazone (Actos™)) and their metabolites. The specification also teaches and exemplifies synthesis of capture compounds and provides working examples showing capture and analysis of captured compounds. Other exemplary capture compounds that present drugs for analysis, are described throughout the disclosure, such as in the text reproduced above.

As noted above, the embodiments that presently are claimed are those in which Y is user selected and is a small molecule drug or other moiety listed in claim 1 that interact with non-targets of the drug whose interactions are assessed and non-targets identified. The overall process as claimed is depicted in Figure 30, which shows that a capture compound is mixed with a sample containing a mixture of proteins that are targets and non-targets. Proteins or in a sample with an affinity for the Y (*i.e.*, drug) are allowed to come to equilibrium with the Y function. The X moiety in the capture compound is then activated (for example, with electromagnetic radiation) forming a radical, which is short-lived, to covalently capture the proteins for which Y had an affinity. Proteins are not captured if the capture compound was not in very close proximity due to the equilibrium between Y function and such proteins. The capture compounds with captured protein are isolated or separated

from the sample via the Q moiety, for immobilization or other separation/identification, and the captured proteins can be identified, such as by mass spectrometry, to identify non-targets that interact with Y.

The results in Figures 31-38 demonstrate reduction to practice of the method as claimed. Figure 31 shows selective protein capture using capture compounds that present drugs whose interactions with a sample are assessed. Capture compounds A and B (page 124 of the application) contain a sulfonamide (a drug) that interacts with Carbonic Anhydrase (CA). Capture compound A presents a 4-sulfamoyl benzene carboxamide. Sulfonamides, such as these, are known inhibitors of carbonic anhydrase (CA), and are used as topical anti-glaucoma drugs. CAII is the target; CAI is non-target. Example 14 and Figures 31-38 demonstrate use of this compound in the method as claimed. These figures demonstrate isolation of CAII (target) as well as CAI (non-target) from complex mixtures, indicating that a capture compound, that presents a drug, such as a benzene sulfonamides not only bind to the targeted CAII, but also bind to non-target, related molecule, CAI, which could be a source of side-effects of these drugs.

The assays described in Example 14 were performed to determine the Kd. According to literature, the Kd of the sulfonamide for the CA II isoform is ~10 nM, and for the CA I isoform is ~1 uM. These values were independently confirmed using the assay as described in the Example, in which the capture compounds are incubated with a sample containing the CA isoform under conditions that allow it to reach equilibrium. The CA isoform is then captured using X. Using purified proteins, affinity and capture efficiency is highest for CA II, lower for CA I, and negligible for other purified proteins tested.

Figure 32 shows relative binding strengths of protein isoforms to a known ligand for capture compound B. Figure 33 shows isolation of Carbonic Anhydrase from complex protein mixtures using capture compound A. CA II was doped into a FPLC purified protein mixture from the human kidney cell line HEK293, The doped CAII was pulled out from all other proteins using avidin-coated (SoftLink) resin. Other proteins were discarded, yielding purified protein ready for further analysis. Figure 33 shows incubation of a capture compound that comprises a drug with a sample of purified proteins from a cell line that contains the target protein. The CAII was isolated and detected. Thus, Figure 33 shows practice of all steps of the method:) contacting a capture compound with user-selected Y groups with a sample for a sufficient time for the interaction between the capture compounds and the biomolecules to reach equilibrium, (2) activating X to form a covalent linkage or high

affinity bond with a biomolecule to effect capture thereof; and (3) identifying captured molecules is described and exemplified in the application.

Figure 34 shows isolation of Carbonic Anhydrase from a highly complex protein mixtures using capture compound A. CA II was doped into the whole cytosolic extract from the human kidney cell line HEK293, . The doped CAII was pulled out from all other proteins using avidin-coated (SoftLink) resin. Other proteins were discarded, yielding purified protein ready for further analysis. Figure 34 also shows practice of all steps of the method.

Similarly Figures 35-38 show practice of all steps of the method. Figure 35 shows capture and isolation of Carbonic Anhydrase from lysed red blood cells. The top spectrum in the figure shows direct MALDI of lysed red blood cells (no purification) wherein signal for Hemoglobin, which is in huge excess over all other proteins, can be seen. Signals are seen for the alpha and beta chains, and also for non-specific dimers (~30 kiloDaltons). The bottom spectrum in the figure is taken after capture compound A, containing a sulfonamide drug with an affinity for Carbonic Anhydrase, is mixed with the lysed red blood cells. The capture compound covalently captures the Carbonic Anhydrase isoforms I and II. All other proteins that are not covalently captured, including nearly all of the Hemoglobin which is in 2-3 log excess, are washed away prior to MALDI analysis. No gel or chromatographic cleanup is required to obtain this spectrum. The intensity of the CA II peak is higher than CAI (which is more ~100x more abundant in RBCs) because the sulfonamide drug has a higher affinity for CAII.

Figure 36 shows direct capture of Carbonic Anhydrase from red blood cells, without pre-lysis of the cells. Figure 37 shows capture of Carbonic Anhydrase from red blood cell lysate when unbiotinylated proteins including Carbonic Anhydrase are in huge excess. Figure 38 shows capture of proteins with lower affinities using very high concentrations of capture compound A.

Thus, these figures show that capture compounds that present drugs can be used to assess their interactions. Figures 31-38 show reduction to practice of each step of the method:) contacting a capture compound with user-selected Y groups with a sample for a sufficient time for the interaction between the capture compounds and the biomolecules to reach equilibrium, (2) activating X to form a covalent linkage or high affinity bond with a biomolecule to effect capture thereof; and (3) identifying captured molecules is described and exemplified in the application

In addition, Example 16 provides a step-by-step example for identifying structural features that contribute to pharmacologic/therapeutic profile and differences in activity within a structural subclass, such as the thiazolidinediones, which are ligands of the PPAR-γ 2. PPAR-γ 2 predominantly is expressed in adipocytes, intestine, and macrophages and possibly muscle cells. Thiazolidinediones (Glitazones) include the drugs:Troglitazone (Rezulin™) Rosiglitazone (Avandia™) and Pioglitazone (Actos™). The anti-diabetic activity of thiazolidinediones is effected by binding to PPAR-γ (gamma) protein. Structure Activity Relationships (SARs) of thiazolidinediones and crystal structures of and PPAR-α co-crystallized with thiazolidinediones is known in the literature

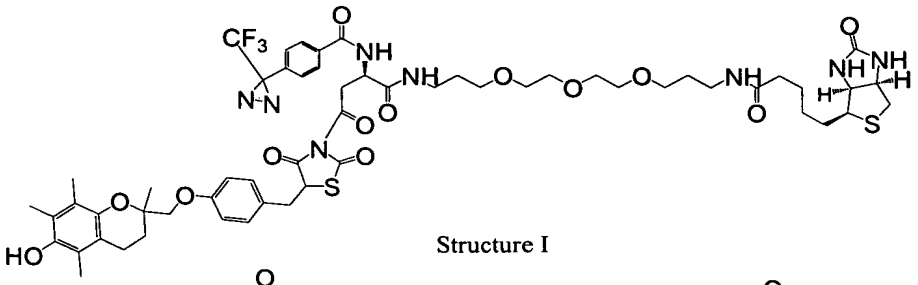
The effect of thiazolidinediones on insulin sensitivity is mediated through altered expression of PPAR-γ 2- dependent genes. Thiazolidinediones, as anti-diabetic drugs, exhibit show toxicity and undesirable side effects. Thiazolidinediones (Glitazones): Troglitazone (Rezulin™) Rosiglitazone (Avandia™) and Pioglitazone (Actos™) are attached Y groups that are attached to Z moiety in the capture compound. These are designated CC-Thiazolidinediones. These are incubated with kidney, liver, pancreatic, colonic epithelium and muscle cells, which are cells in which PPAR-γ is expressed. Rezulin, Avandia and Actos will capture PPAR-γ, PPAR-α and also any non-target proteins with which each interacts. Since these three drugs have different metabolism and pharmacokinetics, they will capture different non-target proteins.

Since undesired and toxic side effects of each of the thiazolidinediones can be due to interaction with PPAR-α and non-target proteins, identification of the captured non-target protein for each drug will provide insights into possible sources of side-effects. In addition, as described and claimed, the drugs then can be modified to eliminate these interactions. The modified drugs can be screened kidney, liver, pancreatic, colonic epithelium and/or muscle cells using these methods to confirm that the they are more specific for the PPAR-gamma target than the original drugs.

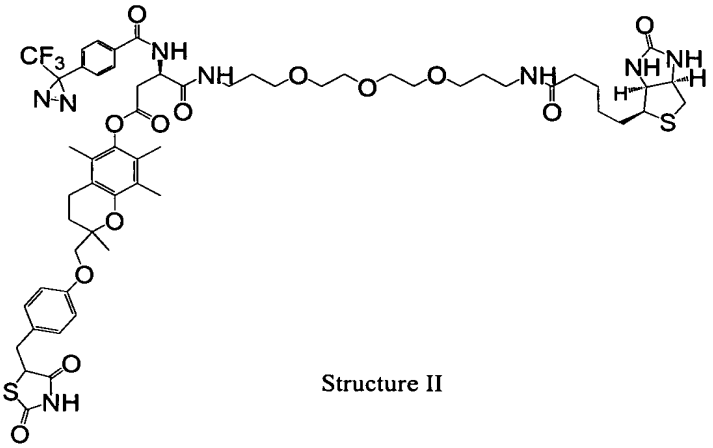
Example 16 describes this and also shows the structures of capture compounds (reproduced below) to be used in the method (incubation with kidney, liver, pancreatic, colonic epithelium and muscle cells for time sufficient to reach equilibrium, activation of the X group to capture the interacting molecules, and identification of the captured molecules. The other working examples in the application show that compounds have been prepared and that they will capture molecules and that mass spectrometry can be used to identify captured compounds. Description from Example 16 is reproduced as follows:

Rezulin:

Rezulin is attached to the Capture Compound as depicted below:



Structure I



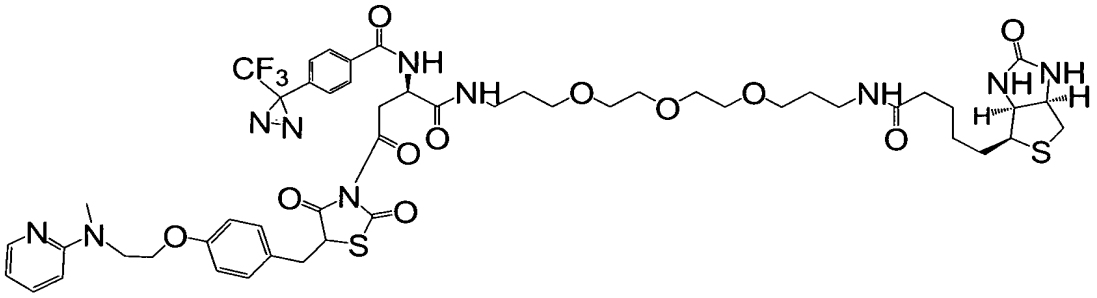
Structure II

Rezulin is metabolized in the liver to its p-Hydroxy glucose and sulfate complexes. Therefore Structure II is used.

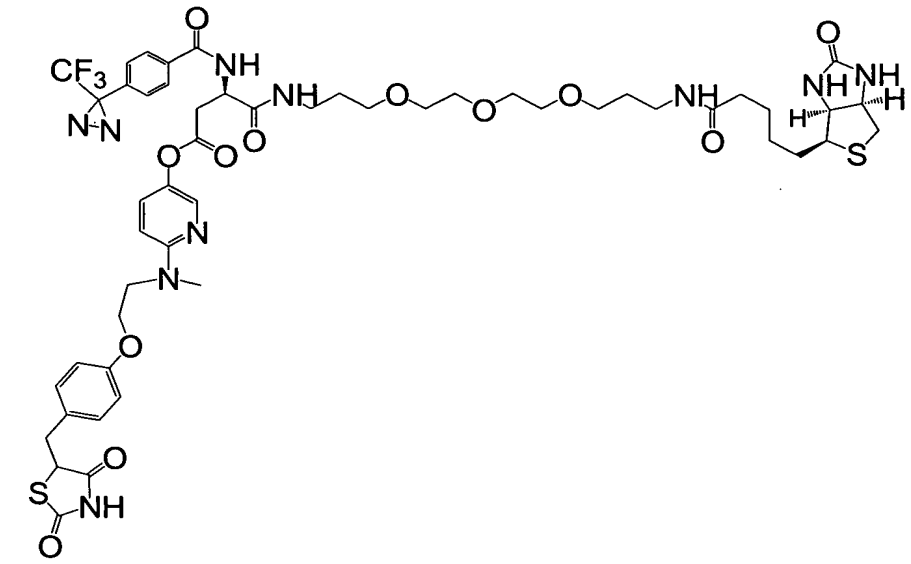
Rezulin Capture Compound Structures I and II are incubated with kidney, liver, pancreatic, colon epithelium, and muscle cells. The target protein PPAR-γ as well as non-target protein PPAR-α and protein A, B and C are captured.

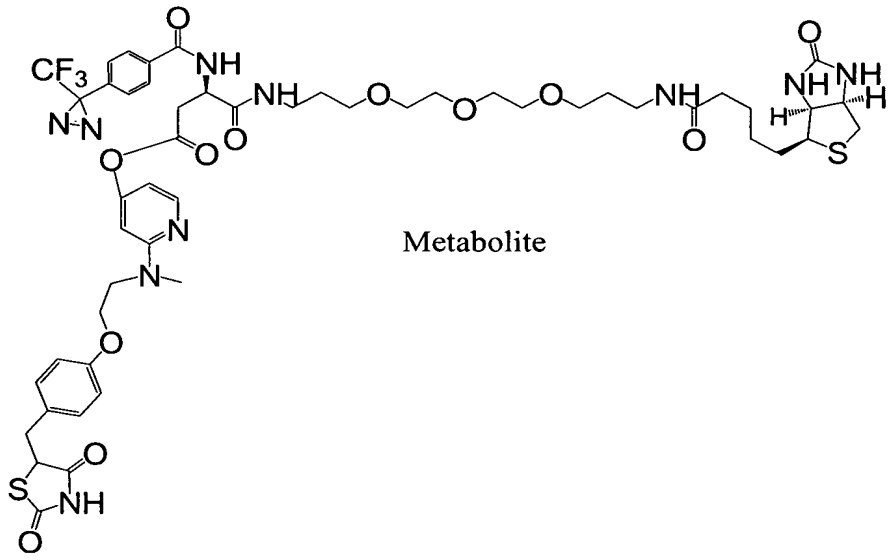
Avandia and Its Metabolite:

Avandia is attached to the capture compound as depicted below:

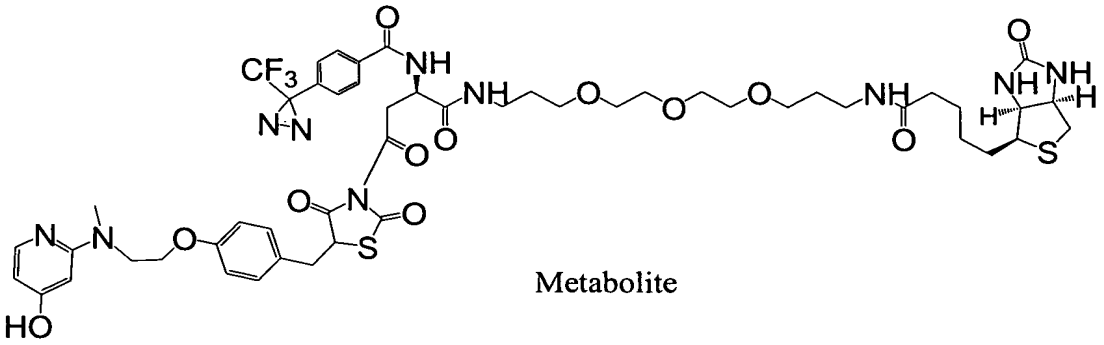


Avandia metabolizes to aromatic hydroxy metabolites. Therefore two possible metabolites are attached to the capture compound as depicted below:

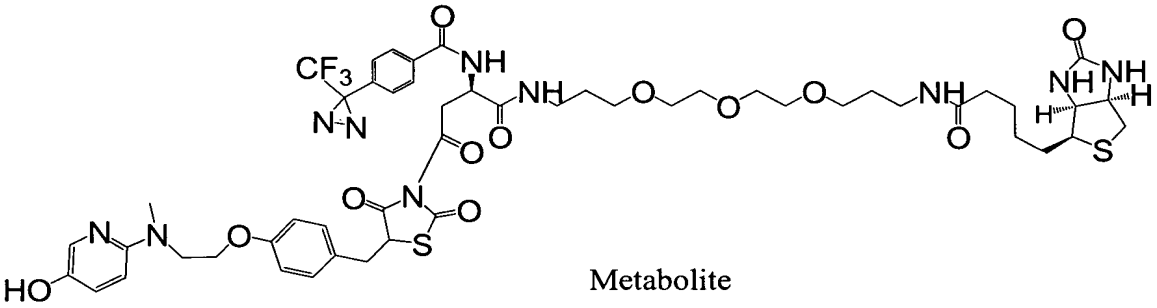




Metabolite



Metabolite

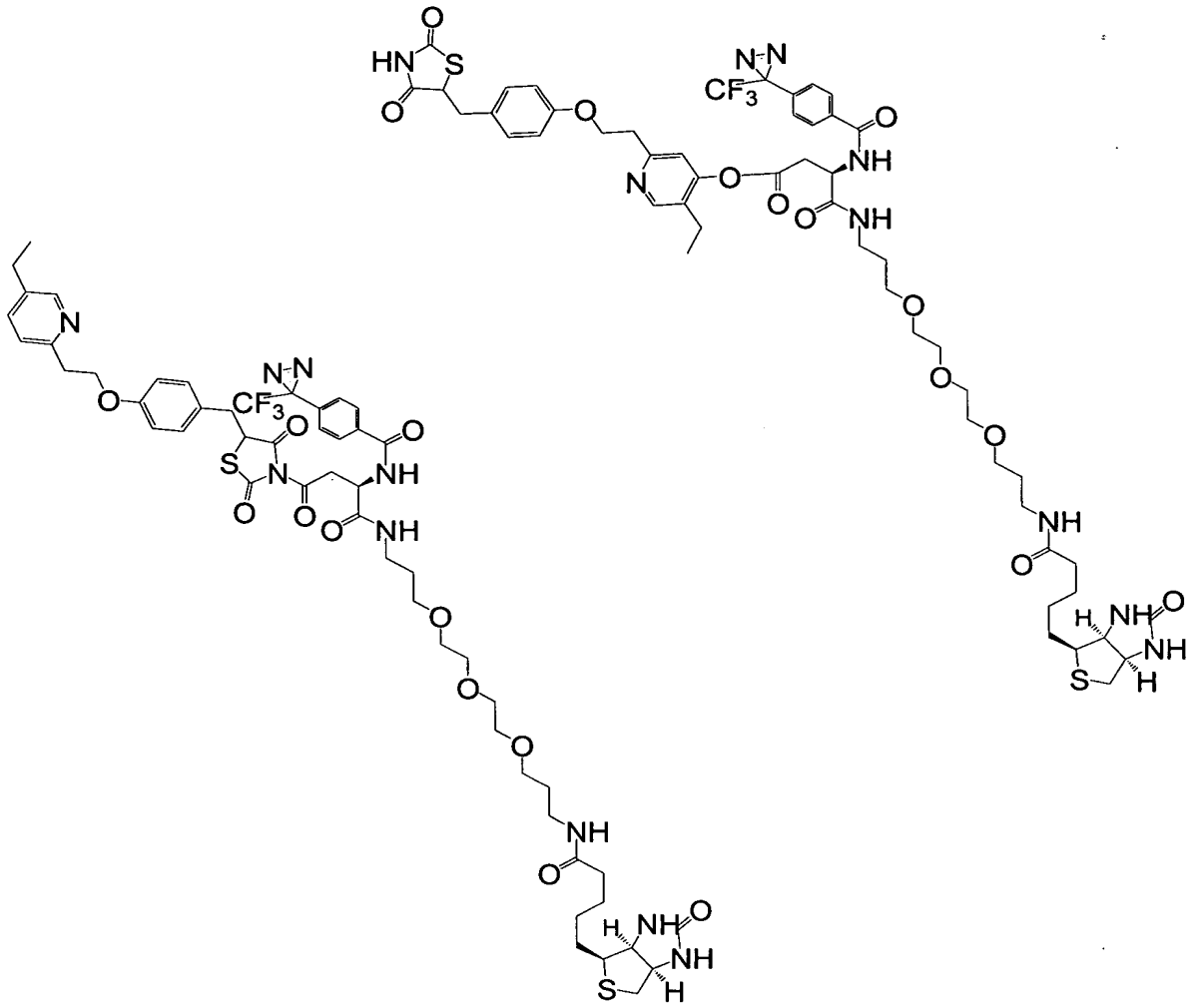


Metabolite

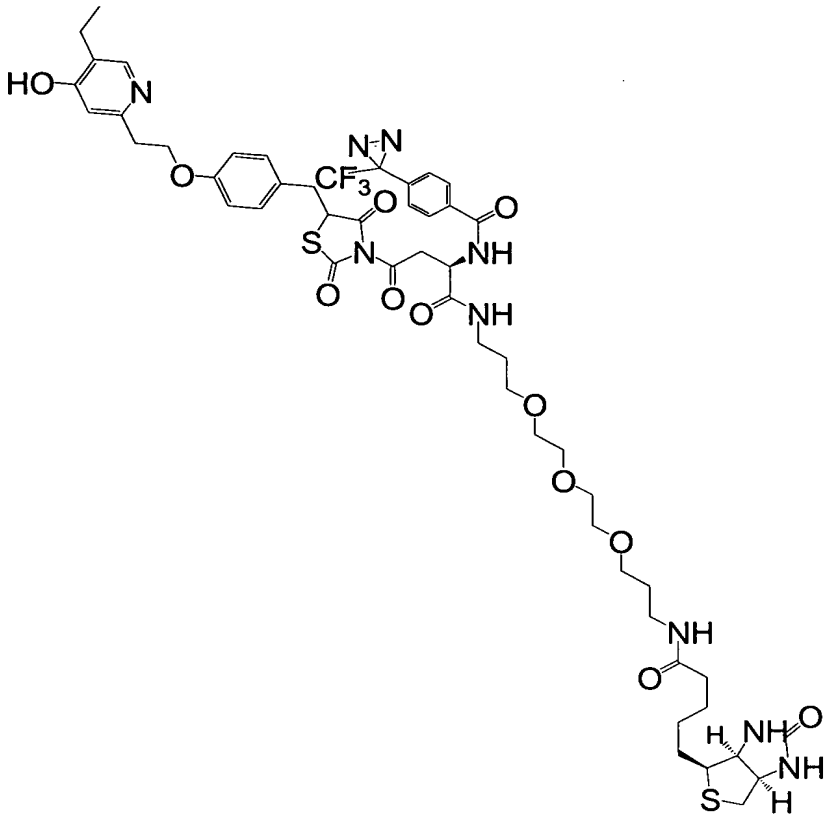
The capture compounds with Avandia and its metabolites attached to the Capture Compound are incubated with kidney, liver, pancreatic, colon epithelium, and muscle cells. The target protein PPAR-γ as well as non-target protein PPAR-α and protein A, B and C are captured.

Actos and Its Metabolites:

Actos is attached to the Capture Compound as depicted below:



Actos' metabolite is attached to the capture compound as depicted below:



Actos and its metabolites attached to the Capture Compound are incubated with kidney, liver, pancreatic, colon epithelium, and muscle cells. The target protein PPAR-γ as well as non-target protein PPAR-α and protein A, B and C are captured.

Thus, there is detailed description in the application, the original claims, description in the Examples, including Examples 14 and 16, and the depiction in Figure 30 and data in Figures 31-38 for practice of method as claimed. Example 14 and Figures 31-38 describe and show practice of steps of the method. Disclosure in the specification describes the method and provides exemplary capture compounds that present a variety of drugs. The specification details exemplary Z, X, Y and Q moieties, including the extensive description in the specification and Figures 16 and 17. Example 16 explains in great detail how an exemplary family of drugs can be studied using the method. Example 16 shows the structure of capture compounds used in the method and also identifies metabolites and capture compounds containing metabolites. The Example identifies cells from which extracts and be prepared and incubated with the capture compounds, as in Figures 33-38. The captured products can be detected as in Figures 33-38. The application teaches and demonstrates how to practice the method as claimed and shows that capture compounds prepared as described can be used

to capture molecules that interact with a Y moiety that is user selected, such as a drug or drug metabolite or drug fragment.

The Examiner urges:

> Applicants set forth only a handful of examples in their specification that have been used to capture compounds under "equilibrium" conditions. For example Q could be biotin, an oligonucleotide, hex-His, antibody, lectin, PNA, peptide(see specification, page 53, paragraph 1). No entirely "inorganic" Q sorting function is disclosed. X, according to Applicants, could be a photoactivatable group or an activated ester if used under acidic conditions (e.g., see also page 87; see also Example 15; see especially page 197, lines 5-11 describing why photoactivatable groups are required, "The central assumption is that the photolysis process is a very rapid process so that the amount of the covalently crosslinked substrate enzyme complex is directly proportional to the amount of the complex in equilibrium"; see also page 47, last full paragraph wherein an azide is presented; see also page 124, compound A for an example of such an azide; see also page 76, paragraph 1 wherein a diazirine group is disclosed and an NHS group that is "inert" under acidic pH but is subsequently activated at high pH; see also original claims 141 and 142 disclosing arylazides and phenyl azides). Although many other X groups were described in the specification, none were described as being able to capture compounds under "equilibrium" conditions. Several commonly known drugs were described for the "Y" position such as Troglitazone, Rosiglitazone, Pioglitazone (e.g., see prophetic example 16) and atorvastatin calcium i.e., LIPITOR (e.g., see specification page 91). A drug metabolite of Actos and Avandia were also described (e.g., see specification, pages 206 and 207). No example of a drug "fragment" is provided that could read, quite literally, on a single carbon atom. No example of a "prodrug" is provided. Finally, only multivalent "carbon based" Z presenting units are provided. No inorganic examples are given (e.g., see claim 34).

As discussed above and throughout this response, applicant is claiming a method to identify non-targets of a small molecule drug. The user of the method presents its a drug (or a fragment, metabolite, prodrug or intermediate of the drug that interacts with a non-target) on a capture compound and practices the steps of the method (as discussed several times above and below). The particular drugs discussed in the application, are discussed to exemplify practice of the method and by no means should the method be limited to assessing only the interactions of these particular drugs. There is no reason why one of skill in the art would not be able to select another drug. Further, the skilled artisan would be able to identify intermediates, metabolites, prodrugs or fragments of the drug, whose interactions would yield insights into the drug non-targets, to present on the capture compounds.

(6) Predictability in the art and the amount of experimentation

Predictability in the art refers to reproducibility of the claimed subject matter. There is nothing of record to suggest that the methods are not reproducible. The methods involve the use of compounds of a defined structure, whose components are well known in the art.

As discussed above, many examples exist of capture compounds that present X moieties for capturing biomolecules. Each step in the method can be practiced without resort to further experimentation. The Y groups are user selected. Regarding predictability, Lauf *et al.*, relied on by the Examiner is not relevant to the instantly claimed methods. There is no need to discover and test new compounds. If necessary, capture compounds that present X groups are known and taught in the application and the Y groups are user selected. There is no need to explore the universe of compounds, but only to prepare compounds as described in the application for any particular drug studied.

The chemistry needed to prepare the compounds, while exemplified in the application, is based on textbook principles that go back years. No new synthetic methods are required.

As noted the specification details how to prepare compounds and lists exemplary X, Z and Q substituents. For the claimed embodiments , Y is a drug or a fragment, intermediate, metabolite or prodrug thereof. The application provides numerous examples of linkage of drugs of interest to capture compounds can be linked to a Z core that also is linked to a photoactivatable group X that covalently binds to proteins, and a Q group for presentation to assess its interactions.

Conclusions

In light of the scope of the claims, the extensive teachings and exemplification in the specification, the high level of skill of those in this art, the working examples, and the extensive knowledge of those of skill in this art, the reproducibility of binding/capture methods, it would not require undue experimentation for a person skilled in the art to practice the methods as claimed. Accordingly, Applicant respectfully submits that full scope of all pending the claims is enabled.

Fairness

Applicant is entitled to claims that are commensurate in scope not only with what applicant has specifically described and exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed. In this instance, applicant has disclosed and taught a generic method of identifying non-target biomolecules (proteins in the claimed embodiments) that interact with a small organic molecule drug by presenting the drug (or a fragment, intermediate, metabolite or prodrug thereof) on a capture compound and contacting the compound with a sample, such as a cell

lysate, that contains non-target, and following the claimed steps of the method to identify non-target proteins of the drug.

It is unfair and unduly limiting to require applicants to limit the claims, when the application clearly teaches how to practice the method as claimed. Once one of skill in the art reads the specification, such person will be able to readily prepare a capture compound that presents the drug (or fragment, intermediate, metabolite or prodrug thereof) on core with an X group for covalent binding to proteins that interact with the drug, and a Q moiety for detecting/separating/immobilizing the capture compounds. The specification clearly places those of skill in the art in possession of a larger genus and teaches how to make and use such genus. To limit the claims to a few species as suggested by the Examiner, is unfair and contrary to the public policy upon which the U.S. patent laws are based. See, for example, *In re Goffe*, 542 F.2d 801, 166 USPQ 85 (CCPA 1970):

> for the Board to limit appellant to claims involving the specific materials disclosed in the examples so that a competitor seeking to avoid infringing the claims can merely follow the disclosure and make routine substitutions "is contrary to the purpose for which the patent system exists – to promote progress in the useful arts."

The public purpose on which the patent law rests requires the granting of claims commensurate in scope with the disclosure. This requires as much the granting of broad claims on broad inventions as it does the granting of more specific claims on more specific inventions. *In re Sus and Schafer*, 49 CCPA 1301, 306 F.2d 494, 134 USPQ 301, at 304. If applicant is required to limit the claims to particular moieties X, Y, Z and Q, then those of skill in the art, by virtue of the teachings of this application, readily can use other moieties X, Z and Q in capture compounds, select a Y, and practice the methods as disclosed in the application, but avoid infringement of claims so-limited. It is particularly unfair to require applicant to limit the method to only testing and indentifying non-targets of particular drugs, mentioned in the specification to depict and exemplify practice of the method. Clearly, the instant application, which teaches to isolate and identify drug targets/non-targets that interact with a small molecule drug, teaches a generic method for doing so. Having done so, the application places the public in possession of such knowledge. Having provided this disclosure, others benefit therefrom. Those of skill in the art should not be permitted to practice what is taught in the application, but avoid infringing the claims. To permit that is simply not fair. Small early stage innovative companies can ill-afford to dedicate their innovations to the public.

Rebuttal to arguments of the Examiner:

The Examiner urges that:

> Applicant's claims are directed to a broad genus of methods for isolating and identifying biomolecules that have been "captured" by a capture compound *of* formula Q-Z-$(Y/X)_{n/m}$. The Q moiety is described as a sorting function, Y is a pharmaceutical drug, drug fragment, drug intermediate, drug metabolite or prodrug. X is a ligand to a biomolecule that binds with sufficiently high affinity so that it will be "stable" under mass spectrometric analysis. And Z is moiety for presenting X, Y and Q. Thus, the claims encompass virtually an infinite number of methods employing virtually an infinite number of capture compounds because no structural limitations have been set forth. That is, Applicants have not limited the number of atoms, types of atoms, or the manner is way said atoms can be connected in defining the Q, X. Y and Z moieties. They could be composed of any element in the periodic table. Furthermore, the dependent claims also fail to limit at least one of the X, Y, Z, and Q moieties to anything less than an infinite number of possibilities. Thus, Applicant's claims encompass the entire universe of drugs, drug fragments, drug metabolites, sorting functions, ligands, etc. without exception. Consequently, the nature of the invention cannot be fully determined because the invention has not been defined with particularity.

Applicant respectfully disagrees. As discussed above, in great detail, the claims are directed to a method comprising contacting a capture compound with a sample comprising biomolecules to effect capture of biomolecules in the sample. The claims recite that Z is a core for presenting X, Y, Z and Q. The application provides detailed description of Z moieties that can be used X, including photoactivatable moieties that covalently binding proteins, the capture moiety is selected to, upon photactivation, covalently bind to proteins, and Q is a sorting function for immobilizing the capture compounds. The specification provides detailed guidance and exemplification of X and Q moieties. In addition, such moieties are well known to those of skill in the art (see, discussion herein). Y is small organic molecule drug or a fragment, intermediate, metabolite or prodrug thereof. As demonstrated herein, small molecule drugs are well known, the skilled artisan, can select one whose interactions with non-targets are to be assessed. The skilled artisan could prepare a fragment, intermediates, metabolites or prodrug if desired, is such were of interest in assessing non-targets. Selection of a Y is a user's choice, since the method is designed to identify molecules with which Y interacts.

The instant application provides the description and teaching for identifying biomolecules that interact with a pharmaceutical drug, drug fragment, drug intermediate, drug metabolite or prodrug by contacting a capture compound with a sample comprising biomolecules to effect capture of biomolecules. The application describes exemplary ways to

identify a drug target (see, e.g. Figures, 20b-e and 33-38; Examples 10 and 14 and pages 151-155 of the application).

The Examiner urges that:

> . . .the predictability in the art is low when the full scope of the claims is taken into consideration. For example, Lauf *et al.* state, "The preparation of new materials with novel and useful chemical and/or physical properties is at best unpredictable considering current levels of understanding. Consequently, the discovery of new materials depends largely on the ability to synthesize and analyze new compounds. Given approximately 100 elements in the periodic table, which can be used to make compositions consisting of three, four, five, six or more elements, the universe of possible new compounds remains largely unexplored." (e.g., see U.S. Patent Application Pub, No. 2004/0062911 Al, page I, paragraph 4). Thus, the presently claimed compounds by analogy "remain largely unexplored" because they could be constructed of any conceivable combination of elements in the periodic table. Furthermore, although organic chemistry (i.e., compounds restricted to a limited number of elements in the periodic table) is a mature art, it is not sufficiently developed to permit the synthesis of any pharmaceutical drug, drug fragment, drug intermediate, drug metabolite. Etc. For example, Keaslin (sic) *et al.* state, "many natural products which would include pharmaceutical drugs, drug fragments, drug intermediates, drug metabolites, etc.) have complex structures, and, as a result, are currently ... impossible to synthesize- (e.g.. see Keasling *et al.*, US Patent Application No. 2006079476, paragraph 6).

The claims are directed to methods for identifying drug non-targets. The methods are not of an infinite nature, but require specified steps. The issue is not whether all possible compounds and drugs are taught in the application or whether all possible combinations of substituents could be synthesized, but whether one of skill in the art, in view of the specification and the other "Wands" factors can practice the method as claimed for a particular drug fragment, drug intermediate, drug metabolite or prodrug of interest. As discussed extensively above, the specification details and exemplifies how to make and use capture compounds and how to practice each step of the method. Those of skill in the art can prepare capture compounds that contain a group Q for sorting/immobilizing and a photoactivatable X group present on a trifunctional Z core

There is no reason to doubt that one of skill in the art could identify targets/non-targets for particular a drug, fragment, metabolite, intermediate or prodrug thereof.

The Examiner further states:

> Applicants assert "[T]he claims are not directed to compounds, but are directed to methods... one [compounds] that is selected by a user to be assessed." However, the instant claims are not drawn to a screening method for identifying "a capture compound", where unknown compounds/molecules are selected. The instant claims are drawn to a method of using "a capture compound" for identifying other targets/non-targets. The requirement under 35 USC 112, 1st paragraph is that "to enable any person skilled in the art to which it pertains, or

> with which it is most nearly connected, to make and use the same..." That is one of ordinary skill in the art must be able to make and use the instant claimed invention. In order to use the instant claimed methods, one of skill in the art must also be able to make and use the "capture compounds," which are necessary to perform the claimed methods.
>
> As discussed above (in the body of the rejection), the state of the art demonstrates (see cited references) that it is highly unpredictable to make and use any "capture compound" that are composed of any chemicals (as it is broadly claimed in the instant case).
>
> Applicants cited several references to indicate the "capture compounds" (Reply, p.20, last para) are known in the art. However, applicants also admit that at least capture compounds containing a "Y moiety" and the X (or the combination thereof) are not described in the listed citations. Therefore, applicants have not demonstrated that it is predictable to generate any capture molecule with any substituent groups including the various claimed components such as the Y, X, Z and Q moieties. In other words, one of skill in the art must by trial and error to combine the various components to arrive at a usable "capture moiety" for selection of a target. This trial and error process has not been demonstrated to be predictable and can be done without undue experimentation. For examples, at least the various chemical reactions that might be involved in forming the capture moiety would be highly unpredictable because different chemical substituent groups would have different reactivity and compatibility with each other. Neither the art nor the instant specification provides a predictable and/or systemic way of generating any capture moiety with any chemical compounds.
>
> Applicants also cited a few working examples of the instant specification. However, these working examples only provide a few chemical compounds that have similar structures, which have not been demonstrated to be generalizable to any compound (such as the ones do not share the same core chemical structures).

The discussion above, establishes that one of skill in the art could readily present a drug of interest on a capture compound, since suitable capture compounds and/or the X, Z and Q moieties therefor are well known and also are detailed in the specification. The Examiner has not provided any reasons why the skilled artisan could not practice the method as claimed. The fact that the prior art does not include a "Y" moiety on capture compounds is because the prior art does not teach the instantly claimed methods; the prior art does not contemplate employing a capture compound to present a drug in a method as claimed This does not mitigate against enablement, since X, Q and Z moieties are known, and the drug will be user selected. The skilled artisan can present a drug on a capture compound.

There is no requirement in the law that the skilled artisan generate any capture compound; rather the test is whether the skilled artisan can prepare a capture compound within the scope of the claims and practice the method as claimed. The requirements of 35 USC §112, first paragraph, can be fulfilled by the use of illustrative examples or by broad terminology. *In re Anderson*, 176 USPQ 331, 333 (CCPA 1973):

> ... we do not regard section 112, first paragraph, as requiring a specific example of everything within the scope of a broad claim .... What the Patent Office is here apparently attempting is to limit all claims to the specific examples, not withstanding the disclosure of a broader invention. This it may not do.

In re Grimme, Keil and Schmitz, 124 USPQ 449, 502 (CCPA 1960) :

> It is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species. It is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it.

THE REJECTION OF CLAIMS 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158-161, 163, 164, 166 AND 169 UNDER 35 U.S.C. §112, FIRST PARAGRAPH- WRITTEN DESCRIPTION

Claims 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158-161, 163, 164, 166 and 169 are rejected under 35 U. S. C. §112, first paragraph, as failing to comply with the written description requirement because the claim(s) allegedly contains subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The previous rejection is maintained for the reasons of record as set forth in the previous Office action as well as for the reasons discussed and rebutted in turn below. Briefly, the Examiner urges that the claims are directed to a broad genus of methods for isolating and identifying biomolecules that have been "captured" by a capture compound of formula Q-Z-(Y/X)n/m. The Examiner alleges that the specification does not define these moieties so that the "claims encompass the entire universe of drugs, drug fragments, drug metabolites, sorting functions, ligands, etc."
The Examiner states that "the method employs molecules with Q, Z. X and Y that can only be distinguished from other compounds by their function." Furthermore, the Examiner urges that the knowledge and level of skill in the art do not supplement the omitted description because no known structure/function relationship and/or chemical properties exists that could otherwise be used to show possession of the enormous genus. This rejection is respectfully traversed.

Relevant Law

The purpose behind the written description requirement is to ensure that the patent applicant had possession of the claimed subject matter at the time of filing of the application *In re Wertheim*, 541 F.2d 257, 262, 191 USPQ 90, 96 (CCPA 1976). The manner in which

the specification meets the requirement is not material; it may be met by either an express or an implicit disclosure.

The Federal Circuit has discussed the application of the written description requirement of the first paragraph of 112 to claims in the field of biotechnology. See *University of California v. Eli and Co.,* 1 19 1559, 43 1398, 1406 (Fed. Cir. 1997). The court explained that:

> In claims involving chemical materials, generic formulae usually indicate with specificity what the generic claims encompass. One skilled in the art can distinguish such a formula from others and can identify many of the species that the claims encompass. Accordingly, such a formula is normally an adequate description of the claimed genus . . . a generic statement such as "vertebrate insulin or "mammalian insulin without more, is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function. It does not specifically define any of the genes that fall within its definition. It does not define any structural features commonly possessed by members of the genus that distinguish them from others. One skilled in the art therefore cannot, as one can do with a fully described genus, visualize or recognize the identity of the members of the genus. A definition by function, as we have previously indicated, does not suffice to define the genus because it is only an indication of what the gene does, rather than what it is.

The court also stated that "[a]written description of an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula, [or]chemical name, 'of the claimed subject matter sufficient to distinguish it from other materials." At 1567, 43 at 1405. Finally, the court addressed the manner by which a genus of might be described. "A description of a genus of may be achieved by means of a recitation of a representative number of species defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus."

The written description for a claimed genus can be satisfied by disclosure of identifying characteristics, including structural and physical characteristics, functional characteristics coupled with known or disclosed correlation with structural characteristics or a combination of such factors sufficient to demonstrate that the applicant was in possession of the claimed subject matter. MPEP § 2163; see *University of California v. Eli Lilly*, 119 F. 3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). Further, as noted above, the standard is an objective one, based on what one of skill in the art would recognize in the

disclosure. *In re Gosteli*, 872 F.2d at 1012. Thus, the knowledge and level of skill in the particular art is a factor to be considered in determining the standard.

The Federal Circuit also has addressed the written description requirement in the context of biotechnology-related subject matter in *Enzo Biochem. Inc. v. Gen-Probe* 296 1316, 63 1609 (Fed. Cir. 2002). The Enzo court adopted the standard that:

> the written description requirement can be met by 'showing that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . complete or partial structure, other physical chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics.' [Emphasis added] at 3.

The court in Enzo adopted its standard from the Written Description Examination Guidelines. 296 at 1324, 63 at 3 (citing the Patent Office's own Guidelines). The Guidelines apply to proteins as well as nucleic acid molecules.

The written description requirement under 35 U.S.C. §112, is distinct from and not coterminous with the enablement requirement:

> The purpose of the 'written description' requirement is broader than to merely explain how to 'make and use'; the applicant must also convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. The invention is, for purposes of the 'written description' inquiry, whatever is now claimed." *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d at 1563-64, 19 USPQ2d at 1117 (emphasis in original).

The issue with respect to 35 U.S.C. §112, first paragraph, adequate written description has been stated as:

> [d]oes the specification convey clearly to those skilled in the art, to whom it is addressed, in any way, the information that appellants invented that specific compound [claimed embodiment] *Vas-Cath, Inc. v. Mahurkar*, at 1115, quoting *In re Ruschig*, 390 F.2d 1990, at 995-996, 154 USPQ 118 at 123 (CCPA 1967).

A specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention, *i.e.*, whatever is now claimed. *Vas-Cath, Inc. v. Mahurkar*, 935 F.2d 1555, 1563-64, 19 USPQ2d 1111, 1117 (Fed. Cir. 1991). A written description requirement issue generally involves the question of whether the subject matter of a claim is supported by or conforms to the disclosure of an application as filed. The test for sufficiency of support in a patent application is whether the disclosure of the application relied upon "reasonably conveys to the artisan that the inventor had possession at that time of the later claimed subject matter." *Ralston Purina Co. v. Far-*

Mar-Co., Inc., 772 F.2d 1570, 1575, 227 USPQ 177, 179 (Fed. Cir. 1985) (quoting *In re Kaslow*, 707 F.2d 1366, 1375, 217 USPQ 1089, 1096 (Fed. Cir. 1983)) (see also, MPEP 2163.02).

An objective standard for determining compliance with the written description requirement is "does the description clearly allow persons of skill in the art to recognize that he or she invented what is claimed." *In re Gosteli*, 872 F.2d 1008, 1012, 10 USPQ2d 1614, 1618 (Fed. Cir. 1989).

The Examiner has the initial burden of presenting evidence or reasons why persons skilled in the art would not recognize in an applicant's disclosure a description of the invention defined by the claims. *In re Wertheim*, 541 F.2d 257, 265, 191 USPQ 90, 98 (CCPA 1976); *See also Ex parte Sorenson*, 3 USPQ2d 1462, 1463 (Bd. Pat.App. & Inter. 1987). By disclosing in a parent application a device that inherently performs a function or has a property, operates according to a theory or has an advantage, a patent application necessarily discloses that function, theory or advantage, even though it says nothing explicit concerning it. The application may later be amended to recite the function, theory or advantage without introducing prohibited new matter. *In re Reynolds*, 443, F.2d 384, 170 USPQ 94 (CCPA 1971); and *In re Smythe*, 480 F.2d 1376, 178 USPQ 279 (CCPA 1973).

Rejected claims

The rejected claims are discussed above.

Analysis

First, to satisfy the written description requirement it is not necessary for the application describe the claim limitations exactly, but only so clearly that one having skill in the pertinent art would recognize from the disclosure that an applicant invented the claimed subject matter. Thus, the fact that the specification does not describe or list all species within the scope of the claim not dispositive of the written description issue. The Enzo court stated that "the written description requirement can be met by that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . complete or partial structure, other physical chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics." 'at 63 at 3 (emphasis omitted, bracketed material in original).

In order to satisfy the written description requirement, one need not provide an example of every species encompassed by a claim. It is sufficient to provide identifying characteristics, including structural and physical characteristics, functional characteristics

coupled with known or disclosed correlation with structural characteristics to demonstrate that the applicant was in possession of the claimed subject matter. MPEP § 2163; see *University of California v. Eli Lilly*, 119 F. 3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). Further, **the standard is an objective one, based on what one of skill in the art would recognize in the disclosure.** *In re Gosteli*, 872 F.2d at 1012. As is discussed in more detail below, it respectfully is submitted that the instant application sufficiently describes the claimed method, including the capture compound reagents employed in the method, to evidence that one of skill in the art would recognize that Applicant had possession of the claimed subject matter at the time of filing and at the earliest claimed priority date.

To satisfy the written description requirement it is not necessary for the application describe the claim limitations exactly, but only so clearly that one having skill in the pertinent art would recognize from the disclosure that an applicant invented the claimed subject matter. Thus, whether or not the specification describes countless capture compounds is **not** dispositive. The Enzo court stated that "the written description requirement can be met by that an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics . . . complete or partial structure, other physical chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, or some combination of such characteristics." 'at 63 at 3 (emphasis omitted, bracketed material in original).

In this instance, the claims are directed to **methods, not to compounds.** The method as claimed is a generic method that can employs reagents known in the art. The method employs capture compounds to identify non-target proteins that have affinity for a drug. This is achieved by presenting the drug (or a fragment, intermediate, prodrug or metabolite of the drug) on a capture compound that includes X, a photactivatable group for capturing proteins, and Q, a function for sorting, separating or detecting the capture compounds. Capture compounds with X and Q groups, as discussed above with respect to enablement are well known t those of skill in the art. In addition, the application describes suitable photactivatable groups and sorting/immobilization/detecting groups in great detail and exemplifies a number of them. The application details selections for each moiety and exemplifies preparation of capture compounds and provides working examples.

As discussed above with respect to enablement and the teachings of the specification examples of each are described. Trivalent Z moieties are described in great detail, with particular formulae. X moieties a listed and include those that are photoactivatable. Q

moieties, including lists of exemplary Q moieties and detailed formulae for some are provided. One of skill in the art can readily appreciate that the application provides a sufficient description to evidence possession of capture compounds for use in the methods.

As discussed above, Figure 30, Example 16, as well as the description in the other Examples and in the detailed description as discussed above, describe the method as claimed. Section C of the application (pages 52-124) describes capture compounds and each moiety X, Z and Q, including examples that meet the requisites of the instant claims. Example 14 and Figures 31-38 describe and show practice of steps of the method. Example 16 explains in great detail how a particular family of drugs can be studied using the method. Example 16 shows the structure of capture compounds used in the method and also identifies metabolites and capture compounds containing metabolites. The Example identifies cells from which extracts and be prepared and incubated with the capture compounds, as in Figures 33-38. The captured products can be detected as in Figures 33-38.

The instant methods, which are new to the art, employ such compounds to present Y, the drug (or a fragment, intermediate, metabolite or prodrug thereof) whose interactions with non-targets are to be assessed. The method recites the steps of contacting the capture compound that presents the Y moiety with a sample, and allowing it to come to equilibrium and then activating X to capture any biomolecules that interact with Y. This is simple textbook chemistry using compounds and moieties that are known. As described and excerpted in detail above, the application describes the methods in great detail, including provision of "detailed, relevant identifying characteristics . . . complete or partial structure, other physical chemical properties, functional characteristics" of the capture compounds that can be used in the methods.

Furthermore, the application details how to prepare and test compounds for the requisite activities. If needed, one of skill in the art, could prepare and test particular capture compounds for use in the method, just as Applicant describes for the exemplified compounds.

Therefore, the combination of the disclosure of the generic structure of capture compounds, the lists of exemplary X, Z and Q moieties, the fact that capture compounds with such moieties are known in the art (see, e.g., Hutchens *et al.* (WO 98/59360), Cravatt *et al.* (WO 01/77668 and WO 01/77684), and Coull *et al.* (EP 0424 819), which are of record in this application), the working examples and generic description of practice of the claimed method, the high level of skill in the art, and the routine nature of the chemistry required

demonstrates that Applicant sufficiently described and was in possession of the method as claimed as of the filing date and priority date of the application.

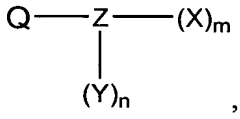
Rebuttal to arguments presented by the Examiner:

(6-) **The Examiner states:**

> the Examiner urges that the claims are directed to a broad genus of methods for isolating and identifying biomolecules that have been "captured" by a capture compound of formula Q-Z-(Y/X)n/m. The Examiner alleges that the specification does not define these moieties so that the "claims encompass the entire universe of drugs, drug fragments, drug metabolites, sorting functions, ligands, etc.

The Examiner states that "the method employs molecules with Q, Z. X and Y that can only be distinguished from other compounds by their function," and urges that the knowledge and level of skill in the art do not supplement the omitted description because no known structure/function relationship and/or chemical properties exists that could otherwise be used to show possession of the enormous genus. Applicant respectfully disagrees

As stated above, the instant claims are to methods in which a user who wants to identify non-targets with which a drug interacts, presents the drug (or a fragment, metabolite, intermediate or prodrug of the drug) on a capture compound that has the formula and structure: Q-Z-X to produce a capture compound:



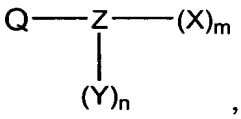
, where m and n are 1.

Q is a moiety for sorting/immobilization or detection of the capture compound; such moieties are well-known and numerous examples are provided in the specification; X is a photoactivatable group tha when activated covalently binds to proteins, and Z, in claim 1, is a trifunctional group for presenting X, Y, Q. As discussed, Z is described in detail in the specification, and Z groups are well known to those of skill in the art as demonstrated above. As stated above, Y **is user** selected. The user identifies a small organic molecule drug of interest and presents it on the capture compound.

The method to which the claims are directed includes the steps recited numerous times above: choosing a small molecule drug whose interactions are to be assessed; providing a capture compound that presents the drug, incubating the resulting capture compound with a sample that contains non-target proteins for a sufficient time to reach equilibrium, activating X to capture the proteins that interact with the drug; and separating or identifying the captured proteins that comprise non-targets. This is **not** "an infinite number of methods employing virtually an infinite number of capture compounds." The claims define the steps

of the method. It is the method that is the "invention" in the instant application; the method in which non-targets are captured by presenting a small molecule drug (or the fragment, metabolite, intermediate or prodrug of the drug) and having a separate X group that, upon activation, captures the proteins that interact with the drug.

The capture compounds do not have an unlimited number of moieties nor an unlimited number of ways that they atoms can be connected. The structure is defined:



, where m and n are 1, and the moieties Q, Z and X are defined (see discussions above) and Y, which is user selected, is small drug molecule who interactions the method assesses to identify drug non-targets thereof. The structure of the capture compound used in the method is defined. As discussed above, while some capture compounds that are used in the methods are novel, capture compounds with Q, Z and X groups suitable for use in the methods are known or can be readily constructed based on the description in the application. The skilled artisan has only to present the drug (or fragment, metabolite, intermediate or prodrug thereof) on a capture compound, whether a known compound of structure X-Z-Q or prepare one as taught in the application, and practice the steps of the method exactly as claimed and described .

(6-) **The Examiner continues:**

> Applicants set forth only a handful of examples in their specification that have been used to capture compounds under "equilibrium" conditions. For example Q could be biotin, an oligonucleotide, hex-His, antibody, lectin, PNA, peptide(see specification, page 53, paragraph 1). No entirely "inorganic" Q sorting function is disclosed. X, according to Applicants, could be a photoactivatable group or an activated ester if used under acidic conditions (e.g., see also page 87; see also Example 15; see especially page 197, lines 5-11 describing why photoactivatable groups are required, "The central assumption is that the photolysis process is a very rapid process so that the amount of the covalently crosslinked substrate enzyme complex is directly proportional to the amount of the complex in equilibrium"; see also page 47, last full paragraph wherein an azide is presented; see also page 124, compound A for an example of such an azide; see also page 76, paragraph 1 wherein a diazirine group is disclosed and an NHS group that is "inert" under acidic pH but is subsequently activated at high pH; see also original claims 141 and 142 disclosing arylazides and phenyl azides). Although many other X groups were described in the specification, none were described as being able to capture compounds under "equilibrium" conditions.

As presently claimed, X is a photoactivatable group that covalently binds to proteins. As claimed in the method, the purpose of X is to covalently capture proteins that are interacting with Y. To ensure that these include non-targets are included X is not activated until the interactions with Y reach equilibrium. As demonstrated above, those of skill in the art are familiar with photoactivatable groups that covalently bind to proteins, and the application, as

recognized by the Examiner provides exemplary groups.

With respect to Q, the specification provides, as noted by the Examiner, exemplary and a representative number of species within the scope of the claims, the art is such that one of skill in the art can select Q for sorting/immobilizing a capture compound. There is no requirement in the law to establish that an Applicant discloses all species within a genus.

(6-) **With respect to "Y," the Examiner states:**

> Several commonly known drugs were described for the "Y" position such as Troglitazone, Rosiglitazone, Pioglitazone (e.g., see prophetic example 16) and atorvastatin calcium i.e., LIPITOR (e.g., see specification page 91). A drug metabolite of Actos and Avandia were also described (e.g., see specification, pages 206 and 207). No example of a drug "fragment" is provided that could read, quite literally, on a single carbon atom. No example of a "prodrug" is provided. Finally, only multivalent "carbon based" Z presenting units are provided. No inorganic examples are given (e.g., see claim 34).

As discussed above, Y is user selected; it is the "test compound" in the method. The examples in the specification are provided to demonstrate practice of the method using exemplary drugs to exemplify a capture compound is used to present a drug of interest for assessing interactions in accord with the method, which, in its generic form is for assessing interactions of small molecules, such as drugs and enzyme substrates to identify non-targets One of skill in the art will select a drug, or make a fragment of the drug, or select an intermediate or metabolite thereof for assessing interactions. The drug fragment does not read on a single atom, as such would not be for assessing interactions as required by the claim. Those of skill in the art can identify drugs of interest. Furthermore, limiting the method to assessing and identifying the interactions of Actos, avandia and Lipitor or a sulfonamide would render the claims unduly limiting. The method is for identifying non-targets of drugs of interest. It makes little sense to limit the claims to assessing interactions of a handful of drugs.

A methods for identifying non-targets of drugs of interest are no different from other methods that assess compounds. For example, methods of drug screening would not be limited to the working examples that identify drugs – there would be no need to ever practice the method again if such were the case. In this instance, the method is for identifying drug non-targets of drugs of interest. The drugs of interest are selected by user of the method. The specification provides examples of drugs that can be used and exemplifies how they are provided on capture compounds for practice of the methods. As discussed in detail above, all steps of the method and how to practice it are described in the application. Again as

reiterated several times above, the instant claims are not directed to compounds, but to methods for identifying non-targets with which a drug of interest interacts. The capture compounds are reagents in the method. As discussed in above, the application describes the reagent (the capture compound) sufficiently to evidence that applicant possessed and appreciated the method as claimed at the time of filing.

4) Regarding the Examiner's comment that the capture compounds include those that present a single atom of a drug because a drug fragment could be one atom, the claims recite that the "the fragment, intermediate, metabolite or prodrug of the drug interacts with a non-target of the drug." Thus, the fragment must be of sufficient size to interact with a non-target with which the drug interacts.

5) The Examiner states:

> To satisfy the written description requirement, an applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the claimed invention (e.g., see *In re Edwards,* 568 F.2d 1349, 1351-52, 196 USPQ 465, 467 (CCPA 1978); see also see also *Vas-Cath Inc v. Mahurkar,* 19 USPQ2d 1111 (CAFC 1991)). Furthermore, a "written description on an invention involving a chemical genus, like a description of a chemical species, 'requires a precise definition, such as by structure, formula [or] chemical name,' of the claimed subject matter sufficient to distinguish it from other materials." (e.g., see *University of California v. Eli Lilly and Co.,* 43 USPQ2d 1398, 1405 (1997), quoting *Fiers v. Revel,* 25 USPQ2d 1601, 1606 (Fed. Cir. 1993)). Here, Applicant has failed to provide a definition, structure, formula or chemical name for at least one of Q, Z, Y and Z describing them in most cases in entirely functional terms. In addition, the CAFC has stated that a genus, which is set forth only in functional terms, "... is not an adequate written description of the genus because it does not distinguish the claimed genus from others, except by function" (e.g., see *University of California v. Eli Lilly and Co.,* 43 USPQ2d 1398, 1406 (1997)). Here, Applicant's claimed screening method employs molecules with Q, Z, X and Y that can only be distinguished from other compounds by their function. For example, claim Q is defined in purely functional terms (i.e., its ability to sort), which was held to be impermissible in *Lilly.* Likewise, Z is defined in terms of its ability to "present" and X is defined in terms of its ability to bind sufficiently such that it is "stable" under mass spectrometric analysis. Just as the generic term "cDNA" did not provide an adequate written description for the broad class of mammalian or vertebrate insulin DNA in *Lilly,* neither does the generic terms X, Y, Q and Z provide an adequate written description for this broad class of capturing molecules because these terms only defines what parts of the compound does (i.e., ability to sort) rather than what it is (i.e., molecular formula such as biotin). In fact, this case is even more egregious than *Lilly* because there is no "genetic code" to correlate the structure with the function. Furthermore, such a correlation could not exist because the claim does not define what is being sorted (for Q), what the conditions for the mass spectrometric analysis are (for X), or what the metabolic/chemical conditions are being considered for the drug metabolite, drug intermediate, prodrug, and drug fragment (for Y)

In order to satisfy the written description requirement, one need not provide an example of

every species encompassed by a claim. **It is sufficient to provide identifying characteristics, including structural and physical characteristics, functional characteristics** coupled with known or disclosed correlation with structural characteristics to demonstrate that the applicant was in possession of the claimed subject matter. MPEP § 2163; see *University of California v. Eli Lilly*, 119 F. 3d 1559, 1568, 43 USPQ2d 1398, 1406 (Fed. Cir. 1997). Further, the standard is an objective one, based on what one of skill in the art would recognize in the disclosure. *In re Gosteli*, 872 F.2d at 1012.

As discussed above, the instant application in view of the high level of skill and knowledge of the skilled artisan sufficiently describes the claimed method, including capture compounds that can be used in the method, to demonstrate possession of the claimed subject matter at the time of the effective filing date of each claim as required by this standard. The application provides detailed examples of each of the moieties, X, Q and Z (Y is user selected), and describes examples of each. The claims recites functional properties of each, including the properties required.

As discussed extensively above, those of skill in the art are familiar with capture compounds that contain X, Z and Q moieties. Those of skill in the art are very familiar with moieties that covalently bind to amino acid side chains, and moieties that require photoactivation for binding. Those of skill in the art know how to label immobilize complexes (Q) and the specification provides a detailed description of such moieties. The claims require Z to be a moiety that is trifunctional for presenting X, Y and Q, and the specification provides detailed descriptions and formulae for Z (see, *e.g.*, pages 54-74,). Dependent claims recite particular Z, X and Q moieties.

As discussed throughout this response, the capture compounds are reagents that one of skill in the art can identify and prepare. The application is directed to a new way of using capture compounds to identify drug non-targets by presenting the drug (or a fragment, metabolite, prodrug or intermediate of the drug that will interact with non-targets of the drug) on a capture compound. One of skill in the art would read the disclosure of this application and recognize that Applicant had possession of the method at the time of filing and at the earliest claimed priority date.

THE REJECTION OF CLAIMS 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158, 159, 160, 163, 164, 166 AND 169 UNDER 35 U.S.C. §102(b)

Claims 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158, 159, 160, 163, 164, 166 and 169 are rejected under 35 U.S.C. 102(b) as being anticipated by

Hasegawa *et al.* (Biol. Chem. 1999, 27-1,44, 31713-31719) as evidenced by, Saeed *et al.*, Samanta *et al.* Chao *et al.*, Savige *et al.* Kahne *et al.* and Adams *et al.* for reasons of record in the previous Office Action. In the previous Action, the Examiner stated that Hasegawa *et al.*, allegedly discloses a method for the determination of the binding site on the extracellular domain of guanylyl cyclase c to a heat-stable enterotoxin by contacting a capture compound with a sample comprising biomolecules to effect capture of biomolecules in the sample. The Examiner urges that the capture compound, an Sta analog, which is shown schematically in figure 1 B, allegedly contains a Z core that presents, Q, X and Y. The Examiner urges that the sorting function is Q is biotin-(AC5)2- Gly-Cys-Cys-G1u-Leu-Cys-Cys-; X = phenyl azide (i.e., a group that is selected to covalently bind to biomolecules) with n = 1; Y is Pro-Ala-Cys-Ala-Gly-Cys; Z = NH-CH(CH_2)-CO of the Pap group. The Examiner states that Hasegawa *et al.* discloses contacting the capture compound and the biomolecules for a sufficient time for the interaction reach equilibrium, followed by activation of "X" to capture the guanyl cyclase. The Examiner says that Hasegawa *et al.*. does not "state that the Pro-Ala-Cys-Ala-Gly-Cys segment is a drug or drug fragment" but relies on supporting references to allegedly show that this sequence is part of the Sta enterotoxin (e.g., see Figure 1), which, secondary references allegedly demonstrate is a drug. The Examiner further contends that molecules that bind to this "drug" are identified and isolated:

> "Hasegawa *et al.* disclose I isolating and identifying the captured biomolecules to thereby identify biomolecules that interact with moiety Y (e.g., see Experimental; see also figures 3 and 4 identifying the isolated SPTFIWK sequence).

The Examiner states that Hasegawa "inherently" discloses the method as claimed. This rejection respectfully is traversed.

Relevant Law

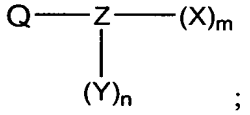
Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada*, 15 USPQ2d 1655 (Fed. Cir. 1990), *In re Bond*, 15 USPQ 1566 (Fed. Cir. 1990), *Soundscriber Corp. v. U.S.* 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), cert. denied, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention." *In re Lang*, 644 F.2d 856, 862, 209 USPQ 288, 293 (CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed subject

matter is disclosed in the reference. *Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984).

When a claimed invention is not identically disclosed in a reference, but requires picking and choosing from among a number of different options disclosed in the reference, the **reference does not anticipate**. *In re Arkley, Eardly, and Long*, 455 F.2d 586, 172 USPQ 524, 526 (CCPA) 1972). Picking and choosing is improper. Rejections under 35 U.S.C. §102 only are proper when a reference clearly and unequivocally discloses the claimed subject matter or directs those skill in the art thereto without any need for picking, choosing and combining various disclosures in the reference. See *In re Le Grice*, 49 CCPA 1124, 301 F.2d 9333. To anticipate the reference must place the public in possession of the claimed subject matter.

Rejected claims

Independent claim 1 is directed to a method for identifying non-targets of a drug that includes the steps of:

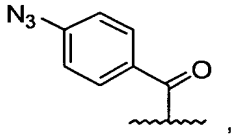
> A method for identifying non-targets of a drug, comprising:
>
> (a) selecting a **small organic molecule drug** whose non-targets with which it interacts are to be identified, and providing a capture compound that presents the drug or a fragment, intermediate, metabolite or prodrug of the drug whose non-targets are to be identified, wherein:
>
> the fragment, intermediate, metabolite or prodrug of the drug interacts with a non-target of the drug;
>
> the capture compound has the formula:
>
> 
>
> ;
>
> X is a photoactivatable group that, upon exposure to light, covalently binds to proteins to effect covalent binding of the capture compound to a protein;
>
> Y is a the pharmaceutical small molecule organic drug Y is the small molecule organic drug or a fragment, intermediate, metabolite or prodrug thereof for assessing interactions with non-targets;
>
> Q is a sorting function for immobilizing or separating the capture compounds;
>
> Z is a trifunctional group containing 50 or fewer atoms that presents X, Y and Q;
>
> m is 1 ; and
>
> n is 1 ;
>
> (b) contacting the capture compound with a sample containing proteins, wherein contacting is effected under conditions in which X is not activated and for a sufficient time for interaction between the capture compounds and proteins in the

sample to reach equilibrium, whereby Y interacts with any drug target and any drug non-target proteins in the sample;

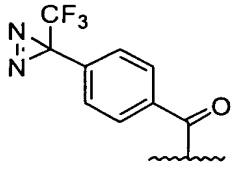
I exposing the capture compound to electromagnetic radiation that activates X, whereby X forms a covalent linkage with protein(s) in the sample that are interacting with Y to effect capture thereof; and

(d) isolating and identifying the captured proteins, wherein the captured proteins comprise non-targets of the drug.

Dependent claims recite particulars of the method, including particular capture compounds and additional steps. For example claims 2, 6, 10, 46, 163, 164 and 175 are recite specific groups for Z, X and/or Q on the capture compounds used in the methods; all require that Z is an amino acid and recite particular groups for Q. For ease of reference claim 2 is directed to the method of claim 1 where Z comprises an amino acid; and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid and an activated dextran. Claim 44 recites that X is a diazirine, 3-trifluoromethyldiazirine or an azide; Z is an amino acid and Q is biotin. Claim 6 recites that X is selected from among an azide or a diazirine; Z is an amino acid; and Q is biotin or an oligonucleotide.

Claim 175 is directed to the method of claim 1, where X is 

,



or an arylazide; Z is serine, threonine, lysine, tyrosine or cysteine; and Q is biotin or an oligonucleotide. Claim 43, recites the method of claim 34, which defines Z, and states that M is an amino acid; S1 and S2 each is independently (CH2)r, where r is 1-10; and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid and an activated dextran. Claim 139 recites the method of claim 137, where M is an amino acid; and Q is selected from among biotin, (His)6, 4,4-difluoro-4-bora-3a,4a-diaza-s-indacene (BODIPY), an oligonucleotides, a nucleoside, a nucleotide, an antibody, an immunotoxin conjugate, an adhesive peptide, a lectin, a liposome, a peptide nucleic acid and an activated dextran; and

claim 140 recites that X is an azide, S1 and S2 each is independently (CH2)r, where r is 1-10, and Q is biotin or an oligonucleotide.

All claims are limited to embodiments in which the user selected Y is a small organic pharmaceutical drug, or a fragment, metabolite, prodrug or intermediate thereof. As noted, Y is user selected; it is the drug whose interactions the method assesses to identify non-targets. Y is presented on a capture compound: X-Z-Q.

Analysis

Hasegawa *et al.*

Hasegawa *et al.* is directed to a study designed to identify the Sta (heat stable enterotoxin) binding region on the extracellular domain (ECD) of guanylyl cyclase. Hasegawa *et al.* specifically affinity labels the binding region for Sta ligand on the ECD of the guanyl cyclase by residue in the Sta with an amino acid analog that is photoactivated to covalently bind. The Sta is contacted with the ECD of guanyl cyclase, and it binds to its binding region, the affinity label is activated so that the Sta is covalently bound to the binding region. The ECD can be digested and the amino acid residues with which the Sta specifically binds are identified. Specifically, Hasegawa *et al.* discloses use of a photoaffinity labeled analog of heat-stable enterotoxin: Biotinyl-$(AC_5)_2$-[Gly^4,Pap^{11}]STp(4-17). (STp (4-17) is porcine Sta with amino acids 4-17.) It is an analog of STp because it incorporates Pap moiety (*p*-azidophenylalanine), which is an amino acid analog, at position 11 and a biotin moiety at the N-terminus. Thus, in the ligand, **X** is the enterotoxin analog since it is the moiety that upon activation covalently binds to the ECD , and biotin is Q. There is **no Y group or Z group**.

This protein ligand is reacted with the extracellular domain of guanyl cyclase to indentify **the region of the extracellular domain of guanyl cyclase to which** the ligand STp binds. After covalently binding this ligand analog to the guanyl cyclase extracellular domain, the resulting complex is digested with Lys-C and the labeled residues identified by mass spectrometric analysis. There is no step in which a Y group is allowed to interact with a sample that contains non-target molecules; there is no separate X group that covalently binds to proteins captures what is interacting with Y and there is no Z group that presents, X, Y and Q. The X group is part of the alleged "drug," the enterotoxin, which cannot be divided up as the Examiner urges. The STp molecule binds to GC; the affinity ligand includes all interacting amino acids. There is no identification or possibility of identification of non-targets of anything, since photoaffinity labeling specifically labels a target. There is no

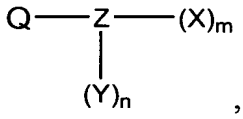
description that for assessing what other molecules besides GC with which the enterotoxin interacts.

Differences between the disclosure of Hasegawa *et al.* and the instant claims

1) Hasegawa *et al.* does not disclose a method comprising a capture compound where Z is a moiety for presenting X, Y and Q as claimed in claim 1.

Biotinyl-(AC5)2-[Gly4,Pap11]STp(4-17) does not have the structure of the capture compounds used in the instantly claimed method.. The biotinyl-(AC5)2-Gly^{4},Pap^{11}]STp(4-17) is an analog of residues 4-17 of Sta and substitutes a p-azidophenylalanine at position 11 so that the Sta will covalently bind to its target in the ECD of guanyl cyclase (GC). First, enterotoxin is not a drug nor is it a small organic molecule drug. Second it is not presented on a capture compound. Third, its interactions are not assessed; the method is for affinity labeling of the binding site for enterotoxin on GC. Fourth, it is bound to its target; interactions with non-targets are not assessed.

In the ligand of Hasegawa, the enterotoxin includes in the portion of the enterotoxin that interacts with the binding site on GC, an amino acid analog that can be photoactivated to covalently bind to the site to which the enterotoxin binds. Thus, the enterotoxin with PAP corresponds to the X group (or to a combined X-Y group) since the portion of Y (the enterotoxin) that interacts with the target ligand, also includes the photoactivatable moiety. As set forth in the claim, X is presented on Z and is not the same as Y nor is it part of the drug or drug fragment.



, where m and n =1.

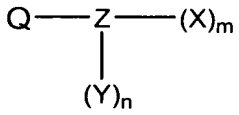
In the enterotoxin ligand of Hasegawa, the enterotoxin portion of the ligand, which includes the PAP moiety, specifically binds to a site on the target binding site in GC and then the PAP group is activated to covalently bind to the site with which it is interacting. The enterotoxin is **not** a small molecule drug. The "X" moiety in Hasegawa *et al.* is not one that covalently captures proteins; rather it is part of the enterotoxin that specifically binds to the target because it is directed to the target by Y. Thus, the "capture compound" used in the photoaffinity labeling method of Hasegawa *et al.* has the structure **X-Q**, where the X group would not capture non-target molecules.

As discussed below in more detail, the Examiner has alleged that somehow, that part of the enterotoxin is Y and X is Pap and Z includes part of Y. The Examiner urges that the

ligand of Hasegawa *et al.* is a capture compound that presents the drug as required in the instant methods. For this interpretation, Z has to include all or part of Y or Q has to include biotin has to include a part of Y (part of the enterotoxin). The structure of the capture compound used in the instantly claimed methods does not allow for this structure, each moiety as defined and exemplified is a separate and distinct from the others. The Z moiety does not include part of the drug. Furthermore, as amended for clarity, the claims render it clear that the drug is not a polypeptide.

In no configuration of the ligand of Hasegawa *et al.* is there a trifunctional moiety, Z, that presents X, Y and Q, nor is there any configuration in which Y and X (the Pap) are separate, since the Pap group is part of the enterotoxin (the "drug") that binds to the active site in GC. In addition, the biotin moiety is linked to the enterotoxin portion of the ligand, so even if X and Y are considered separate somehow, the structure of the ligand is Q-Y-X. or Q-Y(fragment)-X-Y(fragment). If the enterotoxin is considered a drug, then [Gly4,Pap11]STp(4-17) must be Y. There is nothing to conclude that only the STp portion corresponds to Y and the Pap11 portion includes X and the Gly4 portion is part of Z or Q. The claim recites that Y is a drug or drug fragment that binds to non-targets of the drug. There is no disclosure or indication in Hasegawa *et al.* that the STp(4-17) portion binds to non-targets of enterotoxin. Furthermore, Z as defined in the claims is a core for presenting Y, X and Q. Nothing in the definition, description in the application, nor structure indicates that Z includes part of the drug. There is no core moiety that presents Q, X and Y, where X and Y are separate groups and Z is separate from X, Y and Q.

Thus, NO matter how the molecule of Hasegawa *et al.* is construed, there is no Z that separately presents each of Y and X and Q as required in the instant methods, nor is there an X group that is separate from the Y group, since X is part of the binding molecule. No matter what the interpretation, the structure of the ligand in Hasegawa *et al.* cannot be:



as required in the instant claims. X and Y cannot be linked by a disulfide bond, because Z must be trifunctional, and part of the presented drug Y cannot be part of Z or Q. There simply are not four separate components in the ligands described by Hasegawa *et al.* In addition, as required by the claims Y is a small molecule drug, not a polypeptide.

2) Furthermore, the instant claims are directed to **methods, not to compounds**. The method requires contacting the capture compound that presents the drug, which the claim recites is a pharmaceutical small organic drug, not a polypeptide, with a sample that contains **drug non-targets for the purpose of identifying drug non-targets**. Hasegawa *et al.* describes identifying the binding site in the target for the ligand by contacting the ligand with target and identifying the site on the target to which the ligand binds; no non-targets are in the sample. Furthermore the instant claim requires "determining the identity of captured proteins, wherein the captured identified proteins comprise non-targets of the drug." Not only does the method of Hasegawa *et al.* not result in capture of non-targets (since the sample does not include any), the method does not include a step in which identity of captured non-target proteins is determined

Hasegawa does not assess the interactions of the enterotoxin, rather identifies the binding site on its known receptor (guanyl cyclase). Thus, Hasegawa *et al.* does not disclose a method in which the interactions of a small organic molecule pharmaceutical drug (or any molecule) are assessed, nor does Hasegawa *et al.* describe identifying other molecules with which enterotoxin interact. Hasegawa *et al.*. describes affinity labeling and identifying the binding site of a enterotoxin, not a drug, in GC. Thus Hasegawa cannot "inherently" disclose the method as claimed.

3) Hasegawa *et al.* does not disclose a method for identifying non-targets in a sample by reacting the sample with a capture compound. Hasegawa *et al.* discloses a method for photoaffinity labeling the binding site in guanyl cyclase for enterotoxin by contacting the ligand with its known target. No targets are identified; rather the binding site in the target is photo-affinity labeled. No non-targets are mentioned or identified. The reaction mixture includes only the target; it does not include other molecules that have an affinity for enterotoxin.

Thus, Hasegawa *et al.* is probing a receptor to identify the residues on the receptor to which a particular ligand binds. Hasegawa *et al.* does not present a drug Y on a capture compound that contains X-Z-Q, and is not probing a sample to identify drug non-targets. In the method of Hasegawa *et al.*, the target (receptor for the ligand) is known and provided. It is captured because its ligand is designed to specifically only capture the target.

The affinity labeled ligand (the "capture compound") does not interact with non-targets, since the enterotoxin is contacted with its target and no conditions are described in which it would react with a non-target. Hasegawa *et al.* discloses a probe for photoaffinity labeling a predetermined protein: the extracellular domain of Guanylyl Cyclase C (ECD6H). Hasegawa *et al.* prepared biotinyl-(AC5)2-Gly^{4},Pap^{11}]STp(4-17) for photoaffinity labeling of ECD6H" (see, e.g. page 31715, left column, last full paragraph of Hasegawa *et al*). Even assuming that enterotoxin is a drug (which Applicant **does not** concede and certainly is not a pharmaceutical drug nor a small molecule), Hasegawa *et al.* does not disclose a method that identifies with which a drug, drug fragment, drug metabolite, drug intermediate or prodrug interact. The captured molecule in the experiment of Hasegawa *et al.* is the target and it is **known; it is not identified**. In contrast, in the instantly claimed method, drug non-targets <u>are **identified by the method**</u>. Therefore, Hasegawa *et al.* does not disclose a method of **identifying** proteins (non-targets) that interact with a small organic molecule drug or a fragment, intermediate, metabolite or prodrug thereof.

The method of Hasegawa *et al.* for labeling the binding site in GC for enterotoxin, does not capture or identify drug non-targets. It is a method of photoaffinity labeling the binding site in the target. Therefore, Hasegawa *et al.*, does not disclose a method that include <u>**any** **elements**</u> as claimed and cannot does not anticipate claim 1, nor any claim dependent thereon.

Regarding claims, such as 2, 6, 10, 46, 163, 164, 175 and others that specify that Z is an amino acid, it is clear that Hasegawa *et al.* does not employ a molecule that includes Z, and it certainly does not employ a capture compound in which an X and Q groups and a small organic pharmaceutical drug or a fragment, metabolite, intermediate or prodrug are presented on an amino acid.

Therefore, Hasegawa *et al.*, does not anticipate any pending (or previously pending) claim.

Rebuttal to comments of the Examiner:

1) The Examiner states that Hasegawa *et al.* "inherently" discloses the claimed method. As part of this reasoning, states that in the capture compound, which is shown schematically in Figure 1:

> [t]he sorting function Q = biotin-$(AC_5)_2$-Gly-Cys-Cys-Glu-Leu-Cys-Cys-; X = phenyl azide (i.e., a group that is selected to covalently bind to biomolecules) with n = 1; Y = Pro-Ala-Cys-Ala-Gly-Cys; Z = NH-CH(CH_2-)-CO of the Pap group.

This does not comport with the descriptions of Q and Y. In this strained interpretation, Q includes part of the so-called "drug" or "drug fragment." Pro-Ala-Cys-Ala-Gly Cys is part of the enterotoxin that interacts with the target; it is part of the drug. The structure proposed by the Examiner is Q-Yfragment-Z (X)-Yfragment. The claims clearly recite that Q is for sorting and immobilization and Y is the user selected drug or drug fragment. This molecule as defined by the Examiner includes two "drug" fragments. Furthermore, as amended for clarity, the claims recite that the drug is an small organic molecule drug; it is not a polypeptide.

Thus, the capture compound used in the method of Hasegawa *et al.* is not the same as the instantly claimed compounds.

2) The Examiner states, quoting the MPEP:

> When the PTO shows a sound basis for believing that the products of the applicant and the prior art are the same, the applicant has the burden of showing that they are not." *In re Spada*, 911 F.2d 705, 709, 15 USPQ2d 1655, 1658 (Fed. Cir. 1990). The Office does not have the facilities to make such a comparison and the burden is on the applicants to establish the difference. See *In re Best*, 562 F.2d 1252, 195 USPQ 430 (CCPA 1977) and *Ex parte Gray*, 10 USPQ 2d 1922 1923 (PTO Bd. Pat. App. & Int.).

The Examiner is reminded that the claims are directed to **methods**, not to compounds. As discussed above, not only are compounds used in the methods different, the methods are different. As discussed above, the instant method identifies non-targets with which an organic molecule drug interacts, and it requires contacting the capture compounds that present the drug (fragment, metabolite, intermediate or prodrug thereof) with a sample that contains non-targets. In the method of Hasegawa *et al.* the ligand is contacted with its receptor in order to identify the residues in the receptor to which the ligand binds. Hasegawa *et al.* does not disclose identifying any targets and clearly does not identify non-targets of enterotoxin, since the only molecule that it binds to is the ECD of GC.

3) The Examiner continues:

> For claim 2, Hasegawa *et al.* disclose a non-specific target like Tris (e.g., see page 31714, column 2, paragraph 1). In addition, fragments like PTFI and FIWK were also identified in addition to the SPTFIWK (e.g., see page 31716, column 2, last paragraph).

The ligand of Hasegawa *et al.* only interacts with the ECD of GC. Following capture, the ECD in the complex was digested to see what residues in the ECD interact with the enterotoxin ligand. These are not non-targets. Regarding Tris, it is added as a scavenger to inhibit "non-specific cross-linking" of the ligand (and as a buffer). Tris is not a non-target. As discussed above, non-targets are proteins with which the Y drug interacts (see, *e.g.*, the Declaration and accompanying paper and definition and discussions in the application).

4) The Examiner urges that Applicant interprets "the entire amino acid chain including the Pap compound (from residue Gly to the last Cys residue) as the "drug" or the "Y" group because the disulfide linkage." Applicant respectfully disagrees. As discussed above, the claims define Y as the drug (or drug fragment) and the portion of enterotoxin linked to biotin, is part of the enterotoxin, it is part of the "drug" in the ligand, it is not part of the sorting function. It is included in the molecule as part of the molecule that binds to the active site of the ECD. It cannot arbitrarily be designated part of Q.

Further, the Examiner implies that the specification does not limit Q, Z, X and Y groups to be of specific chemical structures. As discussed above, the instant application defines Q and provides numerous examples of Q. Similarly, the specification defines Z and provides numerous examples of Z. X is defined in the claim to be a photoactivatable group that covalently binds to proteins. Y is a user selected organic molecule drug.

As established in the discussion above, those of skill in the art are familiar with capture compounds that contain X and Q groups, and also Z groups, and can readily select those particularly in light of the detailed discussion in the specification. Certainly, one of skill in the art would recognize that the enterotoxin portion of the ligand of Hasegawa *et al.* includes all of the residues (Gly- Cys-Glu . . . Gly-Cys) in light of the discussion in Hasegawa *et al.*, including Figure 1, which shows the alignment with STp and which describes that this molecule binds to the ECD of GC. Any chemical compound group cannot be X, Z, Q and Y, and certainly part of Y cannot be interpreted to be part of Q.

Furthermore, the method of Hasegawa *et al.* does not identify non-targets of enterotoxin, nor does Hasegawa *et al.* provide a method that identifies non-targets nor is it designed to do so. The method is for identifying the residues in the ECD of GC. The instant application clearly describes and defines non-targets (see extensive discussion above) as proteins with which a drug interacts, but that are not the target of the drug.

THE REJECTION OF CLAIMS 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158161, 163, 164, 166 AND 169 UNDER 35 U.S.C. §103(a)

Claims 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 151, 152, 158161, 163, 164, 166 and 169 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Hasegawa *et al.* I in view of Hasegawa *et al.* II as evidenced by, if necessary, Saeed *et al.* (WO 2006/138571 A2, which is **Not** prior art, and Samanta *et al.* and Chao *et al.* and Savige et al *et al.* The Examiner urges that Hasegawa *et al.* teaches all of the limitations of claims 1, 2, 6, 10, 15, 25, 34, 38, 43, 75, 110, 116, 137, 139, 140, 144, 150, 151, 152, 157, 158, 159, 160, 163, 164, 166, 169 and 173, but for claim 161, Hasegawa *et al.* fails to teach a method for disclose a method for determining a dissociation constant. Hasegawa *et al.* only determined I_{50} values (e.g., see page 3 I 715, column 1, paragraph 2), but this deficiency is provided by Hasegawa *et al*, II, which teaches "the use of calculating $K_{D)}$ values to compare in a quantitative fashion the binding affinity of similar peptides (e.g., see abstract; see also Materials and Methods; see also Results)." The Examiner concludes that :

> It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to calculate the $K_{D)}$ value of the biotinyl-(AC5)2- [Gly^4,Pap^{11}]STp(4-17) molecule as disclosed by Hasegawa *et al.* using the method as disclosed by Hasegawa *et al.* II because K_D values were commonly employed as a tool for characterizing the binding affinity of ligand for a protein target (e.g., see Hasegawa *et al.* II, abstract). A person of ordinary skill in the art would have been motivated to calculate the K_D) because it offers an easy, quantitative method for comparing binding affinities that is universally employed in the field of chemistry/biochemistry. A person of ordinary skill in the art would have reasonably expected to be successful because Hasegawa *et al.* II shows that K_D) values can be calculated for nearly identical peptides toxins against the same GC-C targets (e.g., see abstract; see also Materials and methods).

This rejection is respectfully traversed.

Relevant Law

In order to set forth a *prima facie* case of obviousness under 35 U.S.C. § 103(a), there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (*ACS Hospital Systems, Inc. v. Montefiore Hospital*, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention. Further, that which is within the capabilities of one skilled in the art is not synonymous with that which is obvious. *Ex parte Gerlach*, 212 USPQ 471 (Bd. APP. 1980). Obviousness is tested by "what the combined teachings of the references would have suggested to those of ordinary skill in the art." *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981),

but it cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion supporting the combination (*ACS Hosp. Systems, Inc. v. Montefiore Hosp.* 732 F.2d 1572, 1577. 221 USPQ 329, 933 (Fed. Cir. 1984)). "To imbue one of ordinary skill in the art with knowledge of the invention in suit, when no prior art reference or references of record convey or suggest that knowledge, is to fall victim to the insidious effect of a hindsight syndrome wherein that which only the inventor taught is used against its teacher" *W.L. Gore & Associates, Inc. v. Garlock Inc.*, 721 F.2d 1540, 1553, 220 USPQ 303, 312-13 (Fed. Cir. 1983).

Under 35 U.S.C. §103, in order to set forth a case of *prima facie* obviousness, the differences between the teachings in the cited reference must be evaluated in terms of the whole invention, and the prior art must provide a teaching or suggestion to the person of ordinary skill in the art to have made the changes that would produce the claimed product. See, *e.g.*, *Lindemann Maschinen-Fabrik GMBH v. American hoist and Derrick Co.,* 730 f.2d 1452, 1462, 221 USPQ2d 481, 488 (fed. Cir. 1984). The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ2d 1780 (Fed. Cir. 1992); see, also, *In re Papesch*, 315 f.2d 381, 137 USPQ 43 (ccpa 1963). In addition, if the proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. *In re Ratti*, 270 f.2d 810, 123 uspq 349 (ccpa 1959).

For *prima facie* obviousness of a claimed subject matter to be established, all the claim limitations must be taught or suggested by the prior art. *In re Royka*, 490 F.2d 981, 180 USPQ 580 (CCPA 1974). This bedrock principle of U.S. law regarding obviousness was not altered by the recent Supreme Court holding in *KSR International Co. v. Teleflex Inc.*, 127 S. Ct. 1727 (2007). Furthermore, the Court in *KSR* took the opportunity to reiterate a second long-standing principle of U.S. law: that a holding of obviousness requires the fact finder (here, the Examiner), to make explicit some reason that would have led a person having ordinary skill in the art to modify a known composition in a particular manner and thereby result in the claimed composition. Absent such a reason, the claimed composition would not have been obvious.

The rejected claims

The claims are discussed above.

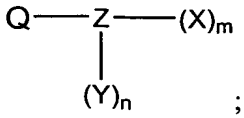
Differences between the teachings of Hasegawa *et al.* and the instant claims

Hasegawa *et al.* is discussed above. As discussed above, it is directed to a study designed to identify the Sta (heat stable enterotoxin) binding region on the extracellular domain (ECD) of guanylyl cyclase. To identify this region, Hasegawa *et al.* employs a photoaffinity labeled analog of heat-stable enterotoxin. The analog incorporates a Pap moiety (*p*-azidophenylalanine) at position 11 in the Sta analog and a biotin moiety at the N-terminus. This ligand is reacted with the extracellular domain of guanyl cyclase and the resulting labeled guanyl cyclase ECD is isolated and digested. The labeled fragment is identified by mass spectrometry to thereby identify the amino acid residues in guanyl cyclase the bind to the enterotoxin.

As discussed above, the photoaffinity labeled analog does not meet the definition of a capture compound (even if one would accept (and Applicant does not) that enterotoxin is a drug) because the Pap moiety is in the middle of the drug inserted at residue 11, and hence the structure of the molecule is Q-Z-Y-X-Y for reasons discussed with respect to the rejection under 35 U.S.C. §102(b). Hence, there is no Z that presents an X, Y and Q. Furthermore, enterotoxin, even if it were a drug, is not a small organic molecule drug; it is a polypeptide.

Notwithstanding such failure, Hasegawa *et al.*, fails to teach, suggest or even hint at a method for identifying drug non-targets (proteins, other than the drug's target, that interact with the drug). Hasegawa *et al.* is directed to a method for identifying the residues in the enzyme to which enterotoxin binds. In the reaction in which the enterotoxin binds, no non-targets are isolated or identified. This is completely and unequivocally different from the instantly claimed method that the identifies non-targets with which a particular drug interacts. There is no suggestion of such method in Hasegawa *et al.* One of ordinary skill in the art, in view of Hasegawa *et al.*, alone or in combination with the secondary references, would not have been led to a method for identifying method for identifying targets and non-targets of a drug by:

> selecting a small organic molecule drug whose non-targets with which it interacts are to be identified, and providing a capture compound that presents the drug or a fragment, intermediate, metabolite or prodrug of the drug whose non-targets are to be identified, wherein:
>
> fragment, intermediate, metabolite or prodrug of the drug interacts with a non-target of the drug;
>
> the capture compound has the formula:

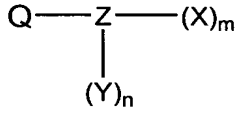


;

(b) contacting the capture compound with a sample containing non-target proteins that interact with Y, wherein contacting is effected under conditions in which X is not activated and for a sufficient time for interaction between the capture compounds and proteins in the sample to reach equilibrium, whereby Y interacts with drug non-target proteins in the sample; and

isolating and identifying the captured proteins, wherein the captured biomolecules comprise drug non-targets.

The capture compounds are of formula:



in which

Z is a moiety for presenting X, Y and Q;

X is selected to covalently bind to biomolecules and requires activation following contacting with the biomolecules to effect covalent binding of the capture compound to a biomolecule;

Y is a pharmaceutical drug, drug fragment, drug intermediate, drug metabolite or prodrug;

Q is a sorting function;

m is an integer that is 1 to 100; and

n is an integer from 1 to 100.

As discussed above, not only does Hasegawa *et al.* not teach a compound of the requisite formula, it does not teach a method that includes a step of identifying drug non-targets. In the study in Hasegawa *et al.*, an analog of a ligand was used to affinity label its receptor in order to identify the residues in the receptor to which the ligand binds. There is no suggestion in Hasegawa *et al.*, nor any other reference of record, to modify the method for identifying protein non-targets which a small organic molecule reacts. There certainly is no suggestion for doing so in order to thereby assess drug interactions for redesign or to identify causes of side-effects.

The mere fact that prior art may be modified to produce the claimed product does not make the modification obvious unless the prior art suggests the desirability of the modification. *In re Fritch*, 23 USPQ2d 1780 (Fed. Cir. 1992); see, also, *In re Papesch*, 315 F.2d 381, 137 USPQ 43 (CCPA 1963). In this instance, there is no suggestion in the cited art for any modification of the study of Hasegawa *et al.* nor any suggestion that would have led one of ordinary skill in the art to the instantly claimed methods.

* * *

In view of the amendments and remarks herein, reconsideration and allowance of the application respectfully are requested.

Respectfully submitted,

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Small Molecule – Protein Interactions

H. Waldmann, M. Koppitz
Editors

With 75 Figures

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Macromolecular versus small-molecule therapeutics: drug discovery, development and clinical considerations

Moo J. Cho and Rudy Juliano

Recent advances in biomedical science in general, and molecular biology in particular, have provided a greater understanding of pathogenesis at the molecular and (sub)cellular level. In turn, this has stimulated the development of macromolecular, mechanism-based therapeutic agents, ranging from recombinant proteins, to oligonucleotides, to genes/gene fragments. The factors essential for the successful development of this new class of therapeutic agents are not necessarily the same as those for the development of conventional small organic molecules. This review mentions several issues relating to the development of macromolecular drugs, and emphasizes the key issue of drug transport and delivery.

Throughout most of the history of modern therapeutics, drugs were equated with small organic molecules. Pharmaceutical chemists both in industry and academia synthesized compounds, usually building upon past successes; biologists then screened these compounds for therapeutic potential. Problems of synthesis, scale-up, analytical methodology, formulation and possible pharmacokinetic and pharmacodynamic behavior were all reasonably well understood. Over the past few years, two distinct, but interrelated, trends in drug discovery have emerged that have drastically altered this situation. The advent of the combinatorial library approach to drug synthesis[1–3] has dramatically expanded the types and numbers of compounds available for testing, thus requiring an equally dramatic increase in the speed and precision required of initial screens[4,5]. However, combinatorial chemistry, like traditional drug discovery, relies heavily on drug screening. A second emerging trend makes use of our increasingly sophisticated knowledge about the molecular pathogenesis of disease processes, and involves designing or identifying macromolecules that interact precisely with required targets for therapeutic intervention. In its broadest definition, macromolecular therapeutics encompasses a number of interesting approaches including antisense oligonucleotides, ribozymes, gene therapy, recombinant peptides and monoclonal antibodies. Each of these approaches has its own advantages, as well as problems; however, as we point out below, they share some characteristics that differentiate them from traditional drug development that relies on small organic compounds.

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Drug discovery

The classic paradigm of drug discovery has been to synthesize a number of organic compounds, test them in a complex, disease-based, screen, identify a lead compound, undertake further synthesis and then re-initiate the cycle (Fig. 1). Promising compounds would then be introduced into extensive preclinical toxicological testing, and suitable compounds would eventually be clinically tested. Recent innovations have dramatically altered the traditional paradigm. First, drug screening is increasingly target based[6–8]. Rather than initially testing a drug in a complex animal model, rapid, high-throughput screens against defined targets (cloned receptors, enzymes, transcription factors) are used to identify promising candidates. Because of the large numbers of compounds that can be tested this way, data handling and analysis become much more complex, so sophisticated information-handling systems are essential[9]. Increasingly, the insights provided by modern molecular biology, particularly genome research, are being used to design strategies for drug discovery[10]. The identification of genes involved in disease processes has made possible a truly rational approach to therapy, whether it involves small organic drugs or macromolecular drugs.

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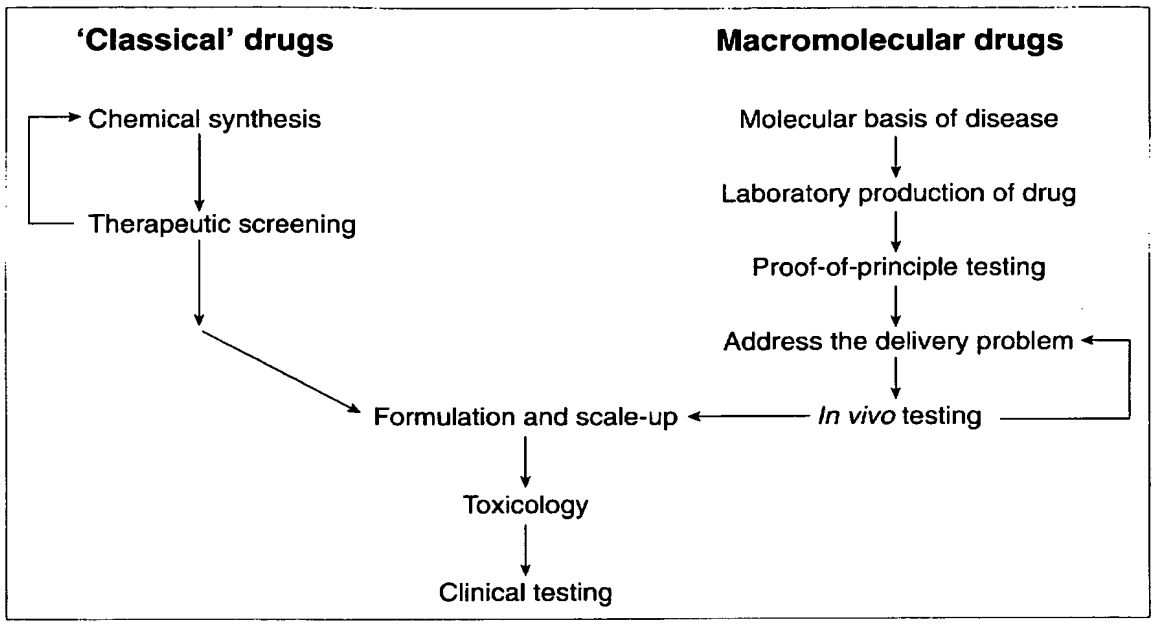

Figure 1

Paradigms for drug discovery. In the discovery phase for 'classic' low molecular weight compounds produced by organic synthesis, a major interactive step occurs between chemical synthesis, as lead compounds are refined and improved, and therapeutic screening (usually in animal models of disease). While scale-up and formulation are important, the transport properties of the drug candidate do not usually represent a major obstacle to further development. Thus, the discovery and development process is relatively simple in structure. For macromolecular drugs, the nature of the therapeutic moiety is defined by an understanding of the pathogenic process. The macromolecule is produced on a laboratory scale, and tested in proof-of-principle experiments where transport and delivery are not an issue (e.g. a therapeutic protein could be directly expressed in cells in culture by transfection with an appropriate vector). For further therapeutic development, the *in vivo* delivery problems must be addressed (e.g. the therapeutic protein may need to be administered by intravenous injection, but the target site is within the cytoplasm of a particular cell type in the body). The iterative process connects the *in vivo* testing of therapeutic efficacy with strategies for macromolecular delivery.

The discovery and development of macromolecular drugs present some unique prospects and problems. For example, many of the recombinant cytokines that are currently undergoing evaluation are highly species-specific. Thus, animal models of efficacy, biodistribution and toxicity are of more limited value than they are for small-molecule drugs, and in-depth human pharmacological studies are required[11].

Using genes as therapeutic agents has challenged the usual pattern of drug development. Genes and vectors are moving from the research laboratory to the stage of clinical testing with less of the extensive preclinical toxicology that has traditionally been associated with the evaluation of low molecular weight drugs[12]. This is not to say that researchers are failing to take suitable precautions to safeguard the welfare of patients. However, the standards concerning the preclinical toxicological evaluation of gene therapy are still evolving[13,14].

Development, production and analysis

The commercial-scale production of recombinant proteins, oligonucleotides and ribozymes, and gene-therapy vectors is a challenging undertaking. The various technologies are currently at different stages of scale-up. The large-scale production of recombinant proteins has made important progress lately[15–17], and is becoming more routine. The automated production of oligonucleotides on solid supports is now chemically straightforward, but there are still scale-up problems[18]. The least-developed production strategies are those for gene-therapy vectors, but these are evolving quickly[14]. Similarly, analytical and quality-control approaches have evolved to varying degrees. The problems with oligonucleotides are perhaps the least severe, as these are at the low molecular weight extreme of the range of macromolecular drugs, and can be effectively analyzed by capillary electrophoresis, or even by simple HPLC. A variety of analytical technologies and bioassays have typically been used at different stages of the development process of recombinant proteins[19], but a major problem still remains the microheterogeneity of protein preparations[20]. However, the advent of powerful mass-spectrometry techniques for proteins promises to provide precise and quantitative analysis of recombinant proteins. Once again, gene therapy lags substantially behind, relying primarily on complex bioassays to establish quality control.

Drug delivery and transport

The discovery of biotechnology-derived macromolecular therapeutic agents is a more directed

process than tended to be the case for conventional organic drugs (Fig. 1), as a result of our increased understanding of disease pathogenesis at molecular and cellular levels. Thus, the main challenge encountered in development is not so much identifying a bioactive molecule but, rather, how to maintain a therapeutically meaningful concentration of the macromolecule in the vicinity of its target for the desired period of time. The intrinsic difficulty arises from the fact that macromolecules are not readily transported across membranes. In recent years, as increasing numbers of macromolecules with therapeutic potential have appeared in the development pipeline, enormous interest has been directed towards understanding macromolecular transport and delivery. Macromolecular transport *in vivo* requires major biological barriers (Fig. 2) to be overcome between the site of initial administration of a macromolecular drug and its action at a target site within the body. Thus, barriers must be crossed in moving from the bloodstream into tissues, from the extracellular space, across the plasma membrane and into the cytoplasm and, in some cases, from the cytoplasm into the nucleus. We will discuss these barriers in the reverse order.

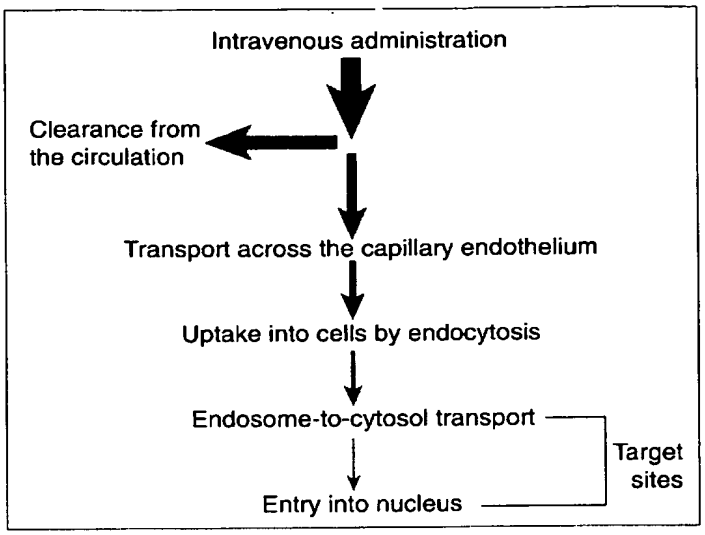

Figure 2

Transport barriers for macromolecular drugs. Subsequent to intravenous administration, a therapeutic macromolecule must overcome several biological barriers to reach its intended target site in the body. Losses occur at each step, as indicated by the arrow sizes. A major goal of macromolecular therapy is to optimize the amount of a therapeutic agent that is delivered to the target.

Cytoplasm to nucleus

The nucleus is enveloped by two layers of membrane that, in places, are fused to each other, creating nuclear pore structures of approximately 70 nm diameter. The pores often occupy as much as 30% of the surface of the nuclear membrane, but their size and number varies depending on the cell type as well as on the phase of the cell cycle. The pores are spanned by a macromolecular assembly known as the nuclear pore complex[21]. The central aqueous channel of the pore complex mediates the free exchange of small molecules, while regulating the vectorial transport of macromolecules in a sieve-like fashion. Measurements with exogenous tracer molecules suggest an effective pore size of 9–10 nm in diameter.

For endogenous proteins or polynucleotides with a diameter greater than 9–10 nm (approximately 40 kDa), diffusional transport via the pore complex cannot account for entry into the nucleus. Instead, the nuclear pore complex facilitates nuclear localization signal (NLS)-dependent, and ATP-dependent, protein import. The NLS is characterized by a short stretch of positively charged Lys and/or Arg residues (e.g. Thr124-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-Glu-Asp-Pro135, which encodes part of the large T antigen of SV40). Thus, with covalently attached multiple copies of an NLS sequence, albumin will enter the nucleus when microinjected into the cytoplasm. The exact location of NLS receptors has yet to be firmly established, but potential candidates, collectively referred to as signal-binding proteins, are found not only on the nuclear envelope, but also in the cytosol. This observation supports the possibility that signal recognition by a cytoplasmic protein is followed by transfer of the signal–protein complex to specific receptors on the nuclear envelope and/or the pore complex[22].

Despite an incomplete understanding of the NLS-mediated transport mechanism, its utility in delivering exogenous macromolecules to the nucleus will no doubt be actively exploited in the future. Small molecules diffuse almost as rapidly in the cytosol as they do in aqueous solution. Together with the permeability of the nuclear pores, this suggests that delivery of small molecules to the nucleus is not a significant problem, as long as the molecules gain entry to the cytoplasm. For macromolecules too big to enter the nucleus by diffusion, NLS sequences may provide a means of facilitating translocation.

Entry into the cytoplasm

Fick's law, as applied to membrane transport, predicts that, for a given membrane thickness, the solute flux is proportional to the diffusion coefficient of the permeant in the membrane, the partition coefficient between the membrane and surrounding medium, and the concentration gradient across the membrane[23]. For spherical molecules, the diffusion coefficient is inversely proportional to molecular radius, and should not vary by more than 20% for typical organic drug molecules that have molecular masses of 300–500 Da. Therefore, the diffusion coefficient is not generally considered an important variable in traditional drug design. However, as the molecular size increases, the diffusion coefficient decreases quite markedly; according to the Stokes–Einstein equation, the ratio of aqueous diffusion coefficients would be approximately 10:3 for two compounds with molecular masses of 300 Da and 1000 Da, respectively.

Little is known about how size affects membrane partitioning. Amino acids have low n-octanol/water

partition coefficients, and polypeptides have even lower values; this has been attributed to a high level of ground-state hydration[24]. In the case of oligonucleotides, the -OH group in the phosphodiester is a strong acid that is negatively charged at physiological pH. The polyanionic nature of oligonucleotides dramatically reduces their partitioning in membranes. This brief assessment demonstrates how difficult it is for macromolecules to enter the cytoplasm by a passive diffusional process, and highlights a significant difference from small organic molecules.

Endocytosis[25] is part of the constitutive trafficking of cell membrane components; it is also an important pathway for cellular uptake of macromolecular drugs. During membrane invagination, molecules are passively internalized as newly generated membrane vesicles are pinched off and enter the cell. The concentration of ligand on the membrane is a major determinant of the efficiency of internalization. In receptor-mediated endocytosis, a ligand is specifically recognized by its receptor, whereas in adsorptive endocytosis, ligand concentration can occur via nonspecific interactions such as adsorption of cationic macromolecules onto the negatively charged cell membrane. A freely soluble macromolecule with no membrane affinity could enter the cell simply by being part of a volume of fluid pinched off into endocytic vesicles.

Molecules entering cells by endocytosis are sequentially delivered to early and late endosomes, endolysosomes and eventually to lysosomes, where they are degraded[26]. In terms of cytoplasmic delivery, therefore, it is imperative to devise means by which macromolecules can escape from endosomes so that intact molecules can be made available in the cytosol. Along the vesicular transport pathway, the intraluminal pH drops as low as pH 5.0, owing to an ATP-driven H^+ pump in the endosomal membrane[27]. This acidification associated with endosome maturation provides the means by which plant and bacterial toxins[28], and certain viruses[29,30] gain access to the cytosol. In both instances, acid-induced conformational changes in the toxin or viral proteins trigger translocation across the endosomal membrane via a fusion process. Attempts have been made to mimic these strategies for the delivery of macromolecular drugs to their cytoplasmic targets. For example, acid-sensitive liposomes containing various phosphatidylethanolamines have long been promoted for drug delivery to the cytoplasm[31]. In addition, peptides that can undergo a structural change from a random coil at a neutral pH to an amphiphilic helix at pH 5.0 can strongly interact with the membrane to induce pH-dependent membrane fusion[32,33]. Therefore, considerable interest has been shown in using pH-sensitive fusogenic peptides, toxin fragments, surfactants and lipids for the delivery of genes[34], antisense oligonucleotides[35] and proteins[36] to the cytoplasm. Despite some success, many questions remain about the mechanism and efficacy of endosome-to-cytosol delivery of macromolecules.

Gene delivery

A particularly challenging delivery problem is implicit in attempts to perform gene therapy. Vectors based on viruses are beyond the scope of this article, but are reviewed elsewhere[37]. Many workers in the field are seeking to use chemical or physical systems for delivering genes. The most popular approach has involved complexing DNA with polycations such as polylysine, or adsorbing DNA onto preformed cationic liposomes; in some cases, peptides and/or proteins designed to promote cell uptake, release from endosomes, or nuclear targeting, are also included[34,38–41]. Despite their popularity, however, the precise cellular and molecular mechanisms of gene transfer by these polycationic agents have not been firmly established. DNA condensation to small submicron particulates appears not only to protect genes from decomposition by nucleases, but also to promote binding to the negatively charged cell surface. A recent study using various microscopy methods has demonstrated that the complexes are generally quite heterogeneous in size and shape, are taken up by the cell via endocytosis, and require dissociation of the complex before genes can be expressed[41]. Once again, the method of escape of the plasmid from the endosomal compartment to the cytosol is unknown.

Drug distribution in organs and/or tissues

The targeted delivery of a macromolecule from general circulation to a specific cell type within an organ is a further challenge. Proteins[42,43] and oligonucleotides[44,45] are either often unstable and/or clear very rapidly from the bloodstream. For example, recombinant human tissue-type plasminogen activator (tPA) has a circulation half-life of a few minutes and is eliminated from the bloodstream by the liver via mannose-receptor-mediated endocytosis[46], and a 25-mer antisense oligodeoxynucleotide phosphorothioate clears with a half-life of 11 minutes in HIV-infected humans[47]. Approximately 60% of a plasmid DNA expressing chloramphenicol acetyltransferase (CAT) is cleared from the blood of mice within minutes, when injected via the tail vein as a complex with cationic liposomes[48]. As is the case for low molecular weight organic compounds, intravenously administered macromolecules are also cleared mainly by the liver and/or the kidneys[49].

There have been a number of attempts to prolong the circulation life-times of injected macromolecules. When several molecules of polyethylene glycol (PEG) are covalently attached to a protein, its circulation life increases significantly[50]. The chemical conjugation of this flexible hydrophilic polymer provides steric hindrance, blocking interactions between serum opsonins and therapeutic proteins or particulate drug carriers such as liposomes[51–53]. In addition to PEG, human IgG1 (Ref. 54), dextrans[55], ganglioside-G_{M1} (Ref. 56), polysialic acids[57] and other macromolecules[58] have been used, with varying levels of success, to extend the circulatory life of proteins and liposomes.

Once a sustained circulation is achieved, target specificity may be built into a construct by means of

antibodies[59,60] or ligands[61–64] to a specific cell-surface receptor that undergoes endocytosis or transcytosis. For example, an 18-mer peptide nucleic acid antisense to the *rev* gene of HIV-1 has been successfully delivered to the brain of rats when it is conjugated, via a biotin–avidin complex, to an antibody against transferrin receptor[64]. Although conceptually feasible, the successful integration of both long-circulation and organ-specific targeting to a satisfactory level has yet to be demonstrated. In a few instances, however, attaching a targeting ligand appears to be sufficient; for example, a plasmid expressing bacterial CAT was efficiently transferred into the liver of rats by asialoglycoprotein–polylysine receptor-mediated endocytosis; cytoplasmic vesicles were shown to be the main site of persistence of the endocytosed DNA, and constructs remaining in circulation were cleared very rapidly[62].

In summary, one of the most challenging aspects of macromolecular therapeutics will be optimizing the delivery of high molecular weight drugs to their target sites within cells and tissues. It seems likely that, in addition to the strategies that have been explored thus far, this will require innovative approaches.

Clinical issues

Extensive clinical experience has been gained with recombinant proteins[11,65–67]. The number of gene-therapy trials has also increased rapidly over the past two years, with most activity being focused on the treatment of cancer[68]. Several clinical trials of antisense oligonucleotides are also in progress[47]. It seems unlikely that the issues and problems associated with clinical trials of macromolecular drugs will be fundamentally different from those involving traditional drugs. Issues of informed consent, patient safety and welfare, enrollment of sufficient numbers of patients to support the trial design, and data accrual and analysis will continue to be the driving concerns of clinical investigators. One issue that may be of key concern is the possibility that the majority of macromolecular drugs could elicit an immune response: not only might this attenuate the therapeutic benefit, but toxicity might result from activation of immune-system cascades.

Summary – advantages and disadvantages

The relative advantages and disadvantages of low molecular weight and macromolecular drugs are quite clear. Standard drugs that are based on organic chemistry offer relatively straightforward and economical approaches to synthesis and scale-up, and their molecular characteristics and purity can be easily assessed with currently available analytical technology. They can readily be designed to cross membrane barriers and so access their targets on, or within, cells. By contrast, macromolecular drugs present far more complex problems in terms of pharmaceutical-scale production, and they are much more difficult to analyze precisely and quantitatively. In addition, formidable transport and delivery problems are associated with macromolecular therapeutic agents. With all of these disadvantages, one might wonder why investigators remain so interested in the prospect of using macromolecules as drugs. The answer lies in the potentially exquisite specificity that one can, at least theoretically, attain by using proteins, oligonucleotides or genes as therapeutic agents. The challenge is to convert the potentiality of macromolecular drugs into practical reality.

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reviews

Engineering challenges in cell-encapsulation technology

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The use of implantable immunoisolation devices, in which the tissue is protected from immune rejection by enclosure within a semipermeable membrane or encapsulant, is one approach in the development of cell therapies. However, further research is required in the areas of: tissue supply from primary or cell-culture sources; maintenance of cell viability and function, its relationship to device design, and the role of, and factors affecting, oxygen-supply limitations; and, protection from immune rejection, especially in view of the mechanisms thought to operate in the presence of a semipermeable membrane, the properties of that membrane, and the implications for biology and device design.

Cell therapy involving the transplantation of cells or tissues with specific differentiated functions shows potential in the treatment of human disease. However, the need for immunosuppressive drugs may lead to a variety of serious side effects[1,2]. One approach to minimizing or eliminating systemic immunosuppression is immunoisolation[3], in which the transplanted tissue is enclosed in a semipermeable membrane in order to protect it from immune rejection, thereby creating what has been termed an implantable biohybrid artificial organ. Devices of this type are under study for the treatment of a variety of diseases, including secretion of insulin in diabetes[4–6], factor IX in hemophilia B (Refs 7,8), human growth factor in dwarfism[9], erythropoietin in anemia[10], as well as for the treatment of kidney failure[11,12], immunodeficiencies[13], and pituitary[14] and parathyroid[15] problems. Potential targets for treatment of disorders in the central nervous system (CNS) include chronic pain[16,17], and neurodegenerative disorders such as Parkinson's disease[18–22], Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis[23]. Treatment of liver failure has

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